Rainfall variability, plant functional traits and productivity in grasslands

By

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Acknowledgements

"I don't know how many years on this earth I've got left. I'm gonna get real weird with it."

-Frank Reynolds

I'd like to thank my wife, Kate Fuller, for her support. My family and friend have always been helpful. I'd also like to thank my committee Sally, David, and Uffe for their comments and encouragement.

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Thesis Abstract

Grasslands are important ecosystems worldwide, and their diversity and productivity is strongly linked to rainfall patterns and soil nutrient availability. Changes in water and soil nutrients, associated with climate change and management regimes, are likely to have strong consequences for grasslands in the future. While the relationship between rainfall, nutrients, and grassland productivity has been well described, a mechanistic explanation as to why some grassland communities are more sensitive than others remains elusive. Plant functional traits are useful to address this issue on a global scale, as they represent life history strategies and are universal across all plant taxa. Such traits often represent the inevitable trade-off between rapidly acquiring resources and the increasing rate of tissue turnover. Such faster-growing species, referred to as *acquisitive* species, can be compared with *conservative* species, which are slower growing and have low rates of tissue turnover, along the resource-use axis. To address how grasslands may respond to future changes in rainfall and fertilisation, I conducted a series of experiments using grasses and grassland ecosystems to study how traits may be used to understand variation in sensitivity across species and communities. My research illustrates that the abundance of acquisitive and conservative plant species in a community can result in high levels of variation in productivity, a primary function of grasslands critical for pasture utilisation and carbon sequestration. I found that root traits of grasses were responsive to phosphorus fertilisation. As the primary organ for water uptake in plants, changes in root morphology associated with P availability may alter how grassland communities respond to changes in precipitation. In addition, I found grouping species based on their functional types was helpful in quantifying plant biomass in remote arid and semi-arid regions, where logistics of scientific investigation can be difficult. More specifically, I developed a set of allometric equations which use non-destructive proxies (plant cover and height) to estimate plant biomass. These estimates were improved by

grouping species based on the functional type (i.e. rough morphological and life-history characteristics). Finally, I investigated how interspecific plant interactions (namely competition) may change under different timing and severity of droughts to identify key candidate grasses for multispecies pasture utilisation. Ultimately, this work contributes valuable information regarding the underlying mechanisms to varying sensitivity in productivity in grassland communities and individual grass species, which will be increasingly important to manage under future climatic scenarios.

Chapter 1: General Introduction

1.1 Introduction to Grasslands

Grasslands account for approximately 29.3% of ice-free terrestrial land-area worldwide (Tanentzap and Coomes 2012; Hooke et al. 2012) and provide myriad ecosystem services including soil stability, crop production, carbon sequestration, grazing opportunities and habitat for many taxa including invertebrates, vertebrates, bacteria and fungi (Daily 1997; Sala and Paruelo 1997; Trumper 2009). The demand for productive and sustainable grasslands to support food production will rise with increasing human population growth (Daily 1997; Trumper 2009). Climate change and land-use change (such as conversion to agricultural systems) are two main threats to grasslands (Bond and Parr 2010), alongside increasing aridity resulting in desertification (Reynolds et al. 2007). Grassland productivity may also be reduced by increased wildfire frequency, high herbivore abundance and subsequent herbivory rates, nutrient depletion/overload, presence of invasive species and other intensive land-use practices leading to degradation (Hoekstra et al. 2005; Suttie et al. 2005).

Grasslands evolved and are maintained by frequent disturbance that prevents long-lived woody plants such as trees from becoming dominant (Suttie et al. 2005; Scott et al. 2010). The composition of grasslands is influenced by abiotic factors including extreme wind, rainfall and temperature, and the dominant grassland species have co-evolved with herbivorous animals such as ungulates, insects and other arthropods (Suttie et al. 2005; Blair et al. 2014). While native grazers are important for grassland maintenance, the introduction of domestic grazing with sheep and cattle, has dramatically altered grassland function and distribution (Vickery 1972; Bailey et al. 1998; Travers et al. 2018). In addition, increasing demand for livestock for meat production (Delgado 2005; Steinfeld et al. 2006; Fiala 2008)

has resulted in over-exploitation of grassland resources (Hopkins and Holz 2006; Machovina et al. 2015). Regular disturbance is common in grass and shrub-dominated ecosystems, but how these systems will respond to drought is important and largely unknown, including mechanisms underpinning grassland-specific variation in sensitivity to drought (Tilman and El Haddi 1992; Nippert et al. 2006, 2018; Guo et al. 2012).

Grasslands support diverse plant communities and species with a variety of life history strategies that encompass many ecological and physiological traits (Christenhusz and Byng 2016). While grasslands are commonly dominated by grasses (Family Poaceae), a variety of other plants with unique ecological niches and life history strategies are also found. These include sedges (Family Cyperaceae), rushes (Family Juncaceae), legumes (Family Fabaceae) and other forbs (Risser 1988). The diversity of species (and associated life history strategies) found in these ecosystems, and their small stature and relatively short life cycles, makes them an excellent experimental model system to investigate the effects of future climate change scenarios on terrestrial ecosystems.

Both natural and managed grasslands are integral to the sustainability and profitability of agriculture worldwide (Tilman et al. 2002; Kemp and Michalk 2007; Klimek et al. 2007). Precipitation is the main climate driver of both the formation and maintenance of grassland ecosystems. Grasslands occur in areas of lower rainfall (200-1000 mm annual precipitation) that do not support trees (Lauenroth 1979; Knapp and Smith 2001) and the majority of grasslands are managed through human activity (Suttie et al. 2005). Management typically occurs through introducing grazers, crop harvest, prescribed fire and herbicide application to prevent woody plant encroachment (Hobbs and Huenneke 1992; Lett and Knapp 2005).

Grassland productivity and rainfall are often well correlated (Sala 2001; Nippert et al. 2006; Yang et al. 2008; Guo et al. 2012). Typically, greater precipitation results in greater aboveground productivity; however, there is also a strong link between variability in

productivity and variability in precipitation (Yang et al. 2008). On a global scale, it has been hypothesised that grasslands with lower mean annual precipitation have more sensitive productivity responses to changes in rainfall because these are primarily water-limited ecosystems (Huxman et al. 2004; Knapp et al. 2015). Conversely, more mesic grasslands (with greater mean annual precipitation), are less limited by rainfall and therefore are more resilient; however, this is not always the case. For example, Guo et al. (2012) observed a greater sensitivity of grassland productivity at sites with greater historic mean annual precipitation amounts compared to those with lower historic rainfall amounts. Others have found productivity is also dependent on long-term rainfall patterns (i.e. legacy effects) (Sala et al. 2012; Reichmann et al. 2013; Griffin-Nolan et al. 2018). Although the precipitation-productivity relationship is fairly well known, the characteristics underpinning these relationships, such as plant diversity and morpho-physiological attributes, are generally understudied (Craine et al. 2013; Skelton et al. 2015; Griffin-Nolan et al. 2018).

Australia is a very large country, extending from 11°S to 44°S, with a variable climate and precipitation ranging from 100 mm to 4,000 mm annually (Fitzpatrick and Nix 1970), supporting different vegetation communities (Suttie et al. 2005). Most of Australia is arid to semi-arid habitat with soils typically of low quality in terms of texture and plant nutrient availability; higher quality soils are generally used for growing crops (Hubble 1970). In Australia, grasslands are a dominant landscape feature and have been used for grazing and hunting over millennia (Suttie et al.2005).

1.2 Grasslands in a Changing Environment

The hypothesis that humans could affect climatic conditions on a global scale was first postulated more than a century ago (Arrhenius 1896). More recently, rising atmospheric concentrations of carbon dioxide (CO_2) and other greenhouse gases (e.g. methane) from

industrialisation have increased air temperatures and the frequency of extreme climate events, such as heatwaves, droughts and floods (IPCC 2018). This poses significant concerns for the future of Earth's ecosystems including grasslands.

Grasslands are considered to be at great risk from global climate change (Sala et al. 2000). The productivity of grassland species is linked to soil nutrients and water availability, with the amount and frequency of rainfall exerting primary control over grassland productivity (Heisler-White et al. 2008; Fay et al. 2011; Walter et al. 2012). It is likely, therefore, that predicted changes in rainfall patterns will have consequences for future grassland productivity, composition and functioning. For example, smaller precipitation events may only wet the upper layers of the soil and increasing temperature and evaporative demands may quickly negate these inputs (Sala and Lauenroth 1982). Alternatively, precipitation inputs of very large magnitude can be detrimental to vegetation, because flooding can lead to root hypoxia, causing reductions in metabolic functions and resulting in mortality (Jackson and Drew 1984; Vasellati et al. 2001). These changes in precipitation can have cascading effects, beyond impacts on vegetation, and affect global nutrient cycles (Nielsen and Ball 2015). Species will vary in their response to changes in precipitation regimes reflecting differences in growth strategies, with further variation mediated by genetic and phenotypic plasticity in relevant plant traits (Volaire et al. 2009; Zwicke et al. 2015; Bristiel et al. 2018). Understanding the mechanisms underpinning drought tolerance across species and how the associated traits will interact with current and future management strategies is key to predicting community- and ecosystem-level response to future changes in rainfall regimes (Mpelasoka et al. 2008; Cullen et al. 2009; Craine et al. 2013; Jung et al. 2014).

Grasses have myriad life history strategies and physiological adaptations to a variety of environments (Christenhusz and Byng 2016). Plant functional traits (*sensu* Reich 2014) are

morphological, physiological and biochemical characteristics that constitute the plant life history or evolutionary strategy, and may also indicate levels of environmental stress. These traits influence the success of a species at a particular site, and can therefore be useful for predicting how species distributions might be shaped by climate and respond to climatic variability (Lavorel and Garnier 2002). Such traits represent evolutionary histories that are widely used to determine competitive ability, acclimation and overall health status of plants worldwide (Diaz et al. 1998; Tilman 2001; Lavorel et al. 2011). A commonly used trait to distinguish between grass species is lifespan (i.e. annual or perennial). This is a useful trait because under drought scenarios, perennial species may outcompete annual species due to more extensive root systems and re-sprouting capacity following foliar dieback (Tilman and El Haddi 1992). Another trait that describes the life history strategy of grasses is whether they undergo vegetative or sexual reproduction. Often, a combination of both reproductive strategies can be utilised as a successful investment of energy towards overall plant fitness. Typically, grasses undergo vegetative (asexual) reproduction as seed germination rates can be low, particularly in more arid ecosystems (Reichmann and Sala 2014).

While the aforementioned traits are useful, a fundamental aspect of trait-based ecology has been how certain traits represent significant trade-offs with respect to acquisition and storage/conservation of resources (Grime et al. 1997; Reich 2014). Thus, plant species possessing certain traits values can be considered as *acquisitive* ("fast and leaky") or *conservative* ("slow and tight") with respect to how a plant utilizes resources (e.g. water and carbon). Such specialisation across this resource-use axis is key to understanding plant diversity, resilience and tolerance to environmental filtering at the organ, individual, community and regional scale (Bello et al. 2013). The abundance and distribution of traits (i.e. functional diversity) within an ecosystem is closely related to the functioning and processes of those ecosystems (Díaz and Cabido 2001; Funk et al. 2017). Therefore, the trait-

based approach to understanding drivers of plant community sensitivity to changes in rainfall may be applied across regions, ecosystems and biomes.

Anthropogenic climate change is causing the environmental filters acting on plant traits to change more rapidly than previously observed (Paoletti et al. 2009; IPCC 2013). Prior to species loss or recruitment, there is often acclimation to the disturbance through intraspecific variation and phenotypic plasticity (Laughlin et al. 2012). Therefore, changes in functional trait assemblages may be instrumental in maintaining ecosystem function and processes. Using the plant functional trait framework is important for addressing which individual traits are likely to be affected by predicted disturbances associated with climate change; however, not all traits are independent (Wright et al. 2004; Westoby and Wright 2006; Wright et al. 2006). For example, high water holding capacity in leaves and deeper rooting depth may be linked because they both contribute to water acquisition and storage. (Grime 2006). In other words, conservative plants tend to have higher tissue densities (mass per area or volume), slow turnover of biomass (high tissue longevity), and slower growth rates. Conversely, acquisitive species have low tissue densities, rapid tissue turnover and higher growth rates. These strategies represent a universal trade-off that exists for all plant species (Reich 2014).

It is postulated that conservative strategies are best during the onset of drought (i.e. declining soil moisture), but less good during recovery periods where rapid growth and establishment are crucial for recovery and/or recolonization following extreme events (Bolger et al. 2005; Volaire et al. 2009; Zwicke et al. 2015). While the abundance of strategies may be important, the historic climatic conditions (e.g. mean annual or inter-annual variation of rainfall) are also important (Yang et al. 2008; Wilcox et al. 2017; Wu et al. 2017). While high inter-annual rainfall variability has been linked to high variation in grassland productivity, the sequence of events is also important for determining changes in species composition and

productivity (Nippert et al. 2006; Reichmann et al. 2013; Griffin-Nolan et al. 2018). Therefore, traits and associated strategies (i.e. *conservative* or *acquisitive*) underpin variation in productivity, and therefore it is important to understand how this might influence grassland productivity in variable rainfall conditions.

Traits have been extensively used in grasslands to understand ecosystem processes and function. For example, Roscher et al. (2012) found that aboveground biomass production in grasses was strongly related to shoot length, leaf distribution and leaf nitrogen concentration, amongst many other traits. Ecosystem processes such as carbon sequestration (De Deyn et al. 2008), litter decomposition (Quested et al. 2007; Fortunel et al. 2009) and grazing potential (Lavorel et al. 2011) are all strongly associated with traits. Traits have been used to understand why plant communities respond to drought events (Comas et al. 2002; Jung et al. 2014; Skelton et al. 2015) and facilitate comparison across experiments and biomes (Díaz and Cabido 2001).

1.3 Current Knowledge Gaps

While the links between grassland productivity and climate have been well-studied (Heisler-White, Knapp, and Kelly 2008; Guo et al. 2012; Wu et al. 2017; Griffin-Nolan et al. 2018), a mechanistic explanation as to why communities vary in their sensitivity of productivity to changes in precipitation remains elusive. Given the predictions of more extreme precipitation regimes around the world (Easterling et al. 2000) including in Australia (Evans et al. 2017), there is a pressing need to develop a mechanistic framework for grassland responses to these events in order to assess future productivity and associated changes in ecosystem services. A primary limitation in our ability to predict how grasslands will respond to changes in rainfall is our lack of knowledge regarding:

1. Species specific responses to changes in rainfall and the underpinning mechanisms (Tilman and El Haddi 1992; Craine et al. 2013).
2. The potential for species' responses to rainfall to be modified by competition from neighbouring species (Callaway and Walker 1997; Brooker 2006; Ploughe et al. 2018).
3. How these changes scale up to the ecosystem level to affect plant community dynamics and ecosystem processes.

The first knowledge gap can be addressed directly by measuring species-specific responses to drought. However, the time and resources required to address such a topic is immense and therefore, a trait-based approach is useful. For example, rather than ask how do species A and species B respond to drought, we could ask "how do *conservative* individuals respond to drought compared to *acquisitive* individuals?" The second knowledge gap is far more difficult to address. However, the importance of alterations in plant-plant interactions is increasingly studied for their relevance to maintenance of ecosystem functions under a changing climate (Smith et al. 2017; Ploughe et al. 2018). For example, if co-occurring species use similar strategies to access water (e.g. deep roots), water limitation can result in direct competition. In deserts, where water is a primary limiting resource, neighbouring species have been hypothesized to use contrasting strategies in order to minimize competition for successful co-existence (e.g. deep rooted next to a shallow rooted species) (Holdo 2013; Ward et al. 2013). This hypothesis, known as Walter's Two-layer Hypothesis (Walter 1971), reflects how resource limitation can affect traits and plant-plant interactions. While the hypothesis was developed as a simplistic explanation for grass and tree co-existence in arid environments, it provides insight into which traits may become important in mesic systems as they become drier. Therefore, although theory can be used to predict how plant-plant

interactions may be affected by drought (see Ploughe et al. 2018 for review), substantial experimental evidence is lacking. The most important uncertainties in predicting future plant community dynamics are:

4. Do individuals and communities respond similarly to soil water limitation?
5. How does the timing and severity of drought affect competition among co-occurring grass species?

Access to grasslands in the wetter portions of Australia (i.e. east of the Great Dividing Range) is generally fairly simple, but access to the more arid regions can be difficult. Only 15% of Australians live further than 50 km from the coast, leading to challenging logistics of work in the interior due to poor infrastructure. While 70% of the land surface of Australia is arid or semi-arid (less than 500 mm of precipitation annually) (Eamus et al. 2016), few people reside in this region despite an abundance of floral diversity (Smith and Morton 1990; Morton et al. 2011). These plant communities contribute greatly to the global carbon cycle, when rainfall does occur, through photosynthesis and biomass accumulation (Haverd et al. 2016; Nolan et al. 2018). However, quantifying biomass production is difficult, particularly because it requires measuring the biomass of all plant species during transient wet periods, and potential changes in plant productivity due to variable rainfall remains largely unknown (Sala and Lauenroth 1982; Austin et al. 2004). Therefore, developing methodologies to rapidly assess and quantify plant biomass in Australia's interior is crucial. The use of allometric estimates has been used world-wide with greater success where biomass is correlated with a non-destructive proxy measurement (e.g. height, plant cover) (Flombaum and Sala 2007; Cleary et al. 2008; Allen et al. 2008; Nafus et al. 2009). Thus, rather than harvesting and transporting large amounts of plant material from remote locations, a simple assessment of cover and/or height can be recorded in the field and used to estimate biomass, following initial calibration of biomass with non-destructive proxy measures on small sample

plots. Additionally, rather than specific relationships between biomass and proxy measures for individual species – which is immensely time consuming and highly site-specific - species can be grouped to reduce the time and cost of estimating biomass in these remote systems. The following questions, however, remain:

6. What non-destructive measurements best correlate with plant biomass (Clifford et al. 2013; Jérôme Chave et al. 2014)?
7. Do multi-species estimates outperform species-specific allometric estimates of aboveground biomass (Nafus et al. 2009; Fayolle et al. 2013)?
8. How do we choose species to group together for such assessments (Nafus et al. 2009; Paul et al. 2016)?

In forests, extensive work has been done using non-destructive measurements such as basal area, wood density and total tree height (Paul et al. 2016). In grasslands, plant cover and height have been found to be useful proxies for productivity (Huenneke et al. 2001; Flombaum and Sala 2007; Nafus et al. 2009; Oliveras et al. 2014). However, while species-specific allometric estimates of biomass have been compared to multi-species estimates in mesic grasslands (Nafus et al. 2009; Fayolle et al. 2013; Ishihara et al. 2015; Paul et al. 2016), little is known regarding arid vegetation (Allen et al. 2008). A final question is how should plant species be grouped to maximize the accuracy of allometric estimates of plant productivity, given that it is rarely possible to obtain such relationships at the species level for all species within a site/landscape? A common method is to group by plant functional types (e.g. trees, shrubs, grasses, forbs, etc.); however, the question of how specific such groupings need to be to accurately predict plant biomass remains unaddressed.

1.4 Thesis Objectives

Based on the identified knowledge gaps, the objectives of my thesis were to:

1. Determine how the abundance of traits affects the sensitivity of productivity in a mesic grassland (Chapter 2).
2. Determine the value of traits as indicators of plant physiological performance in response to extreme drought and soil nutrient management (i.e. fertilisation) in a mesic grassland (Chapter 3).
3. Evaluate the use of plant functional type-based allometric estimates of aboveground biomass in grassland and rangeland ecosystems in remote locations (Chapter 4).
4. Determine how reduced soil water availability, associated with drought, affects species interactions (competition/facilitation) in three important pasture grasses (Chapter 5).

Each of these objectives are addressed in an individual chapter. The first two objectives (Chapters 2 and 3) were addressed in a mesic grassland using a DroughtNet field site (Yarramundi) located at Western Sydney University's Hawkesbury Campus. The third objective (Chapter 4) was addressed at six remote field sites in western New South Wales and southwest Queensland, which are also part of the DroughtNet framework. The fourth objective (Chapter 5) was addressed using the large Rainout Shelter facility located at Western Sydney University's Hawkesbury Campus.

1.5 Data Accessibility

All data, statistical analysis code (R programming) and citations used for this research have been shared amongst my supervisory panel through GitHub (https://github.com/jjchieppa). In addition, data have been uploaded to the Hawkesbury Institute's data management system (HIEv) and archived. For long-term/on-going projects, plant and soil samples have been labelled and stored on campus for potential future analyses.

Chapter 2: Community-Weighted Plant Functional Traits Predict Rainfall Driven Variation in Grassland Primary Productivity

In review with *Oecologia*

AUTHOR LIST: Jeff Chieppa, Uffe N. Nielsen, David T. Tissue, Sally A. Power

AUTHOR CONTRIBUTIONS: All authors conceived and designed the experiment. JC performed the experiment and analysed the data. JC wrote the manuscript; other authors provided editorial advice.

2.0 ABSTRACT

1. The use of plant functional traits to provide a potential mechanistic link between grassland plant communities and aboveground net primary production (ANPP) is crucial for predicting the functional implications of changing rainfall regimes. In this study we used species diversity (richness, evenness) and community-weighted (CW) traits of a mesic grassland plant community in one year (2014) to explain variation in ANPP among 16 plots over the following three years (2015-2017), which was a period of highly variable rainfall.
2. We found species richness and evenness in the initial community to be poor predictors of ANPP in subsequent years. By contrast, CW mean specific root length and specific leaf area were positively correlated with greater variation in ANPP (R^2 = 0.29 and 0.23, respectively). We also used CW trait variances to represent intraspecific variation in traits, finding that the CW variance in specific leaf area, foliar C:N and stem density were all significant predictors of variation in ANPP (R^2 = 0.24, 0.28 and 0.25, respectively).

3. Our results indicate that traits can be useful surrogates for plant strategy along the 'resource-use axis' (i.e. acquisitive vs. conservative) in grassland communities, and that the relative abundance of such traits can predict the stability of productivity in response to changes in rainfall regime.
4. Traits provide mechanistic insight into the relationship between species' resource use strategies and the functioning of mesic grasslands that can be used as a framework for evaluating the impacts of future climate change on a wide range of grasslands.

2.1 INTRODUCTION

Grasslands provide myriad ecosystem services and are important for the sustainability and profitability of agriculture worldwide (Daily 1997; Sala and Paruelo 1997; Trumper 2009). Many studies have examined the relationship between aboveground net primary productivity (ANPP, $g \cdot m^2 \cdot year$) and rainfall in grasslands, finding strong positive relationships across locations world-wide (Sala et al. 1988; Le Houerou et al. 1988; Yang et al. 2008; Wilcox et al. 2017; Knapp et al. 2017). Periods of low rainfall often reduce carbon assimilation and biomass accumulation at the individual and community level (Medrano et al. 2002; Ripley et al. 2010), while higher rainfall can increase productivity via greater carbon and nutrient acquisition (Knapp et al. 2001). However, mechanistic explanations as to why communities differ in their sensitivity to changes in rainfall remain ambiguous (Smith et al. 2009; Knapp et al. 2015). Given predicted changes in the frequency, intensity and size of rainfall events (Easterling et al. 2000; Knapp et al. 2008, 2008; IPCC 2013; Fischer et al. 2013), there is a pressing need to develop mechanistic predictions of grassland responses – both in terms of composition and function - to future rainfall regimes.

The use of plant functional traits has increased our ability to predict and quantify the health and functioning of ecosystems worldwide (De Deyn et al. 2008; Adler et al. 2014;

Skelton et al. 2015). Plant functional traits (*sensu* Reich 2014) reflect species' evolutionary history and are important surrogates for plant strategy (*sensu* Westoby 1998) and performance (Grime 1988; Wright et al. 2004; Funk et al. 2017). The concept that a 'trait' is an important surrogate or proxy for an organism's performance and fitness has been around since Darwin's time (Darwin 1859) and has been substantially developed since (Grime 1988, 2006; McGill et al. 2006; Dwyer and Laughlin 2017). While traits are useful for describing plant strategies (or the niche they fulfil in a community), traits can also represent organismal 'trade-offs' within the context of plant form and function (Wright et al. 2004; Osnas et al. 2013; Reich 2014; Díaz et al. 2016). For example, Grime et al. (1997) identified a 'resource use' axis that prevails across most plant species, which represents an inevitable trade-off between the ability to rapidly acquire resources and the increasing rate of tissue loss (i.e. turnover). Thus, plant species can be described as *acquisitive* ('fast and leaky') or *retentive*/*conservative* ('slow and tight') (Craine et al. 2001; Lavorel and Garnier 2002; Grime 2006). traits are used to describe the position of a species on the 'resource use' axis with respect to neighbouring species (**Table 2.1**).

Table 2.1. List of commonly used plant functional traits, their derivation/units, how they would look in a "conservative" plant species compared to an "acquisitive" plant species, how the trait relates to performance, and references.

Trait (Abbreviation)	Details & Units	Conservative	Acquisitive	Surrogate of Trait Type	References
Specific leaf area (SLA)	Fresh leaf area (mm^2) / leaf dried mass (mg)	Lower	Higher	Relative growth rate (RGR), economic	Poorter and Remkes 1990; Shipley et al. 2006
Leaf dry matter content (LDMC)	Leaf saturated mass (g) / dried mass (mg)	Higher	Lower	Leaf longevity, economic	Ryser 1996
Foliar C:N	Leaf C% / leaf N%	Higher	Lower	RGR, longevity, homeostasis	Wardle et al. 1998; Yu et al. 2015
Vegetative height (Ht)	Ground to tallest leaf base (cm)	-	-	RGR, competitive vigour, root depth/spread	Grime 1988; Westoby 1998; Cornelissen et al. 2003
Stem/tiller density	Count (#) / area (cm^2)	-	-	Regeneration following disturbance	Olson and Richards 1988; Dalgleish and Hartnett 2006
Specific root length (SRL)	Root length (cm) / root dry mass (g)	Lower	Higher	RGR, economic?, foraging potential (FP)	Cornelissen et al. 2003; Kramer-Walter et al. 2016
Root tissue density (RTD)	Root dry mass (mg) / root volume (mm^3)	Higher	Lower	Longevity, economic	(Ryser 1996; Wahl and Ryser 2000; Fort et al. 2015
Root branching intensity (RBI)	Root tips (#) / root length (cm)	Lower	Higher	FP, nutrient supply, potential mycorrhizal colonization	Bardgett et al. 2014; Eissenstat et al. 2015; Zhan and Lynch 2015; Liese et al. 2017; Laliberté 2017
Average root diameter (DIA)	Sum of individual root diameters (mm) / number of roots sampled	Higher	Lower	Hydraulic performance	Comas et al. 2013

The "Biomass-ratio hypothesis" (Grime 1998) states that ecosystem functioning reflects the traits and functional diversity of dominant, rather than subordinate, rare or transient species, and that dominant species' traits are more important than the number or distribution of species (i.e. richness or evenness) (Mokany et al. 2008; Tardif et al. 2014). In order to link the relationship between a traits (or strategy) and the abundance of that trait within a community, the use of community-weighted traits (**Equation 2.1**) has been helpful in testing the Biomass-ratio hypothesis (Garnier et al. 2004). For **Equation 2.1**, the value of a trait ($trait_i$) of a species (i) is weighted by the proportion (p_i) of that species in the community, which is applied to all species (n) within that population.

$$\textbf{Equation 2.1}: Community - Weighted\ Trait = \sum_{i=1}^{n} p_i * trait_i$$

In other words, greater species abundance in a community will exert greater influence through the contribution of its trait to the CWT (i.e. weighted by relative abundance). The actual trait value used in the CWT trait calculation is typically the 'mean' value of a species (CWM), since interspecific differences are considered to be greater than intraspecific differences (i.e. 'mean field theory') (Weiher et al. 2011). However, the role that intraspecific variation plays in community ecology and in mediating responses to environmental perturbation is being increasingly acknowledged (Valladarese et al. 2007; Maire et al. 2009; Jung et al. 2014; Siefert et al. 2015). To account for the potential importance of such intraspecific variation, the use of community-weighted trait variance (CWV) has been proposed (Sonnier et al 2010). Variance, in this case, is the coefficient of variation (CV; standard error/mean), which is unit-less, allowing comparison of CWV from traits that may otherwise be incomparable. The distinction between CWM and CWV is important, as these two metrics of traits in an ecosystem can provide insight into unique aspects of plant strategy (Kichenin et al. 2013). For example, species with high foliar C:N ratios are considered slow

growing, since they are able to accumulate N over a long lifespan (Tateno and Chapin III 1997; Wardle et al. 1998). Alternatively, a narrow foliar C:N range (low CV) across a nutrient or aridity gradient may indicate that a species maintains a consistent photosynthetic rate (consistent N use) and foliar C investment regardless of abiotic conditions (He et al. 2008; Yu et al. 2015). This highlights the unique information provided by a trait mean value and frequency (i.e. dispersion) regarding a species' strategy relative to its neighbours.

The ANPP-rainfall relationship is mediated by the acquisition and transformation of C into the various components of plant biomass (Medrano et al. 2002; Ripley et al. 2010). Therefore, the values of traits that relate to aspects of carbon and water cycling, and their abundance in the community, should predict productivity responses to changes in water availability (**Figure 2.1**) (Wardle et al. 1998; Reich 2014; Skelton et al. 2015). A recent literature review exploring trait-precipitation relationships concluded that these relationships were weak, in part due to poor trait selection (e.g. lack of hydraulic traits,) and the lack of community-weighting of traits (Griffin-Nolan et al. 2018). In addition, the response of ecosystems to variable precipitation will likely be mediated by recent and past rainfall history (Knapp et al. 2008) and the degree of deviation from long term historical conditions (Smith et al. 2009; Smith 2011).

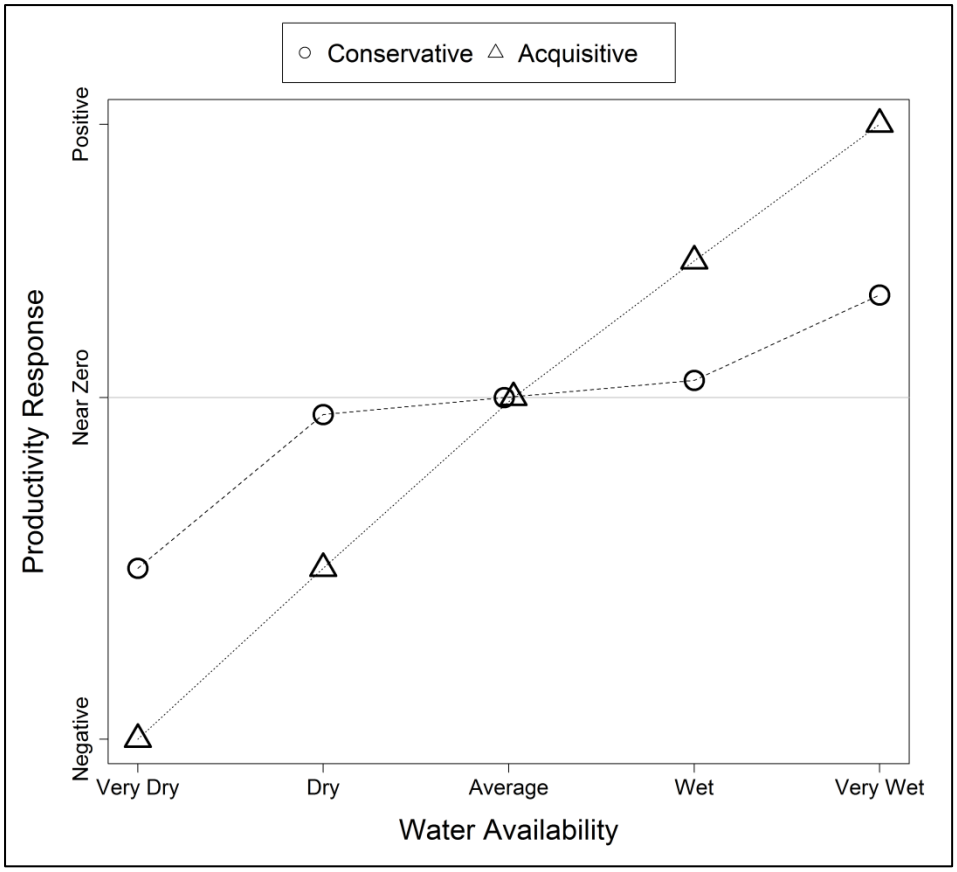


Figure 2.1. The theoretical relationship between changes in ANPP and water availability showing differences between acquisitive and conservative plant strategies. 'Acquisitive' plant species (△) will exhibit strongly positive responses to increasing water availability, but also strongly negatively responses to declining water availability, thereby providing a highly variable contribution to a community's productivity. Conversely, 'conservative' plant species (○) will be less extreme in their responses to changes in water availability, compared to 'acquisitive' plant species. The relative abundance of these different strategies will determine ANPP responses to variable rainfall. In the case of catastrophically low rainfall conditions, most species across the acquisitive-conservative spectrum are unlikely to persist, resulting in a drastic reduction in productivity.

The impact of contrasting traits or strategies on plant productivity in response to variable precipitation has been studied extensively in xeric (water-limited) ecosystems. Walter's "two-layer" hypothesis (Walter 1971) assumes that species competing for a limiting resource will do so by acquiring water in temporally (e.g. annual *vs.* perennial) or spatially (e.g. deep rooted *vs.* shallow rooted) separated ways (Ogle and Reynolds 2004; Ward et al. 2013; Holdo 2013). Additionally, the "pulse-reserve" model (Noy-Meir 1973; Reynolds et al. 2004; Collins et al. 2014) states that 'biologically relevant' rainfall events can stimulate plant growth and reproduction, and are more important than seasonal or annual totals of rainfall as suggested in the two-layer model (Ogle and Reynolds 2004). Both concepts provide intuitive frameworks for understanding dryland plant communities, although support for these paradigms is inconclusive (Belsky 1994; Breshears and Barnes 1999; Reynolds et al. 2000; Reynolds et al. 2004; Maestre et al. 2009). To extend this concept, if a single species exhibits large variation in traits, it may be possible to see some individuals of that particular species respond, while others do not. In mesic grasslands, the absence of woody vegetation can make it appear as if plant form and strategy is convergent (i.e. low variation of trait values across taxa). However, contrasting strategies of resistance and resilience to altered rainfall amounts have been demonstrated amongst co-occurring species in mesic systems (Milbau et al. 2005; Volaire et al. 2009, 2014; Ward et al. 2013; Gibson-Forty et al. 2016) and provide a starting point from which to examine the trait-based mechanisms that underlie relationships between species composition and sensitivity to changes in rainfall regimes across a wide range of grasslands (Volaire 2008; Comas et al. 2013; Jung et al. 2014; Skelton et al. 2015; Barkaoui et al. 2016).

The overall aim of this study was to examine whether traits can provide a mechanistic explanation for inter-annual variation in ANPP in a mesic grassland community. We hypothesized that greater abundance of species with "acquisitive" traits (associated with rapid

growth and low stress tolerance) in the initial community (T_0) would be associated with more variation in ANPP over the subsequent three years (T_{1-3}). Conversely, we expected that a greater abundance of species with "conservative" traits (associated with slower growth) would result in greater resistance (or stability) of ANPP. Similarly, we expected intraspecific variation in traits to be correlated with ANPP rainfall responses, whereby a high proportion of species with broad trait ranges would be associated with high variation (low stability) in ANPP. To compare the use of this trait approach to other commonly used measures of diversity, we also used Shannon's richness, Shannon's evenness and Simpson's richness (T_0) indices to explain variation in ANPP (T_{1-3}). Additionally, we investigated the correlation among community-weighted mean and variance traits, to determine if they are related. Finally, we evaluated the extent of correlation amongst the community-weighted mean and variance traits to investigate whole community trait coordination, as well as coordination within species.

2.2 MATERIALS AND METHODS

2.2.1 Field Site Characteristics and Environmental Monitoring

The field site is located at Western Sydney University's Hawkesbury Campus in Richmond, NSW (-33.61037, 150.741684) at 25 m above sea level. The 100-year (1913-2013) mean annual precipitation is 807 mm (Australian Government Bureau of Meteorology, Richmond – UWS Hawkesbury Station, Station Number 67021). Average summer maximum and minimum temperatures are 29.4 °C, and 18.8 °C, respectively. Winter mean maximum and minimum temperatures are 17.3 °C and 3.2 °C. The soil is Blackendon Sand, with a sandy loam texture (90.2 ± 1.3% sand, 5.6 ± 1.4% clay), and a mineral hardpan at *ca*. 70 cm depth. Soil bulk density at 0-20 cm depth is 1.463 ± 0.039 $g \cdot m^{-3}$. The site was grazed by cattle for

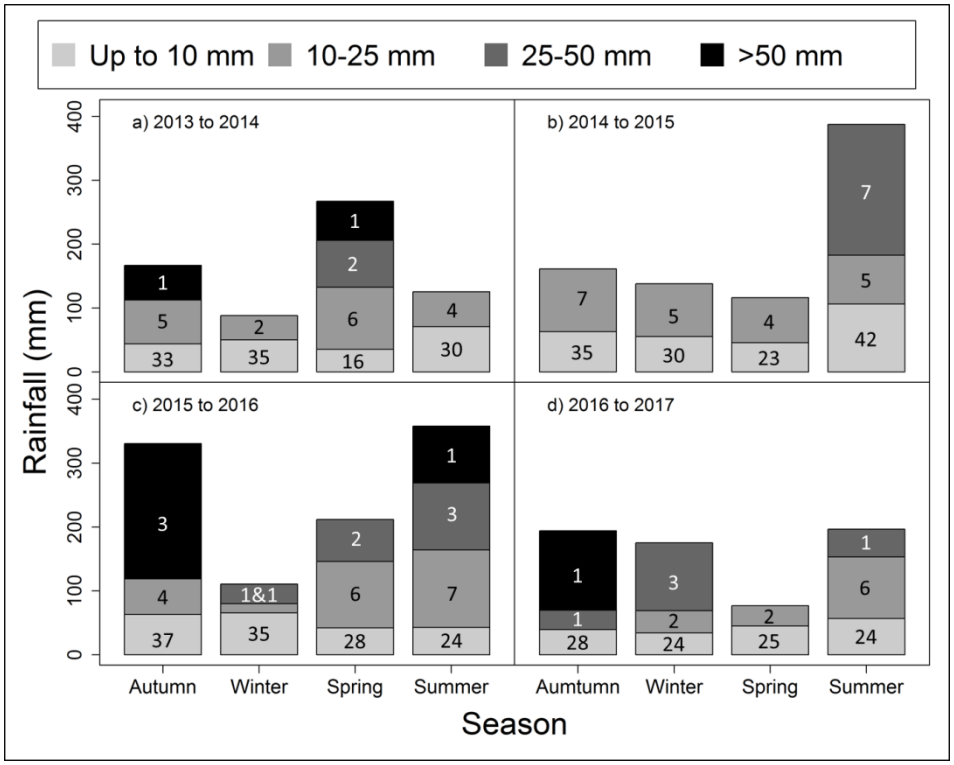
~40 years until 2013 when a fence was installed around the perimeter to exclude large grazers. The most abundant plant species at the site are *Cynodon dactylon* (L.) Pers., *Setaria parviflora* (Poir.) Kerguélen, *Microlaena stipoides* (Labill.) R.Br., *Eragrostis curvula* (Schrad.) Nees, and *Commelina cyanea* R.Br.. *Cynodon*, *Setaria* and *Eragrostis* are all perennial C4 grass species (Field and Forde 1990). *Microlaena* is a native C3 perennial grass (Johnson et al. 2014) and *Commelina* is a native C3 forb (Thorpe 1980). Their contributions to ANPP are described below (see *Community-Weighting and Diversity Calculations* section). From 2014-2018, 22 species were recorded in surveys at the site (**Table S2.1**).

In early 2014, eight plots were established as described in the NutNet protocol (Nutrient Network, http::://www.nutnet.umn.edu/, Borer et al. 2014), with four of the plots receiving phosphorus additions (10 $g \cdot m^2 \cdot$year phosphorus addition) and four (controls) receiving no nutrient additions. In June 2015, eight new plots were established when rainfall shelters were installed. At this time, four of the eight sheltered pots received P addition as described above. Phosphorus was added annually in June throughout the period of the study. Phosphorus was chosen as it was observed to be a limiting nutrient in an adjacent forest experiment (Ellsworth et al. 2017), commonly has sub-optimal availability in pastures in southeast Australia (Nash et al. 2000) and can affect photosynthetic recovery following drought (dos Santos et al. 2006). Rainfall shelters (3 x 3 m) achieve passive reduction of ambient rainfall using clear plastic strips (clear acrylic cast, Perspex) to exclude 65% of ambient rainfall while intercepting minimal amounts of photosynthetic photon flux density. Photosynthetic photon flux density (PPFD) was recorded at 15-minute intervals (Apogee sensors, model SQ-110, ICT International, Armidale, NSW, Australia), beginning on November 11th 2015. The site has two PPFD sensors: one under a rainfall exclusion shelter and another in the open at the centre of the site; light interception by the shelter was less than 10% compared to the open site. The rainfall reduction of 65% was selected based on the most

severe 1-year drought (268 mm in 1944) in the region in the last 100 years. This criterion was required because the site is part of the DroughtNet network (https://wp.natsci.colostate.edu/droughtnet/). The cross of P treatment (no P and added P) and shelter treatment (no shelter and shelter) was replicated across four blocks with one replicate of each treatment combination per block (i.e. 16 plots, 4 in each block).

Three plots (two sheltered and one control) each had two rain gauges installed from 24 April 2016 to 5 October 2016 to determine the efficacy of rainfall interception. During that period, 347 mm of rainfall was captured in the control gauges (~14 discreet events ranging from 1.6 to 147.1 mm), 148 mm in the sheltered plots (i.e. 57.5 ± 5.4% intercepted). Long-term rainfall data were collected at a nearby weather station (~200 m away) at the EucFACE site. In October 2016, the field site/plots were mowed to remove the large accumulation of dead plant material.

In each of the 16 plots, one time-domain reflectometry (TDR) soil moisture sensor (CS606, Campbell Scientific, Thuringowa, QLD, Australia) was installed at a 30° angle to record soil moisture (5 minute intervals since November 11th 2015) in the top 15 cm of the soil. Sensors were located under the shelters in sheltered plots or ~1 m inside the edge of the plot in those without shelters. Soil moisture sensors were checked regularly for accuracy using a handheld soil theta probe (Delta T Devices, UK). Although the rain shelters excluded a substantial amount of rainfall, the effects on soil moisture in the sheltered plots was minimal for the first two years of rainfall exclusion, due to a perched water table across the site and significant summer rainfall (**Figure 2.2**). Soil moisture data indicated that through the growing season (~November to March) leading to harvests in 2016 and 2017, the shelters did not generate a meaningful drought considering the 65% rainfall exclusion shelters in place (**Figure 2.3**).



158

159 **Figure 2.2**. Cumulative rainfall for each season and year, categorised by event size. The
160 number of events in each size class is labelled within seasons. *Note*: In panel C, '1&1' in
161 winter refers to one event in the "25-50 mm" size class and one event for the unlabelled "10-
162 15 mm" class directly below.

163

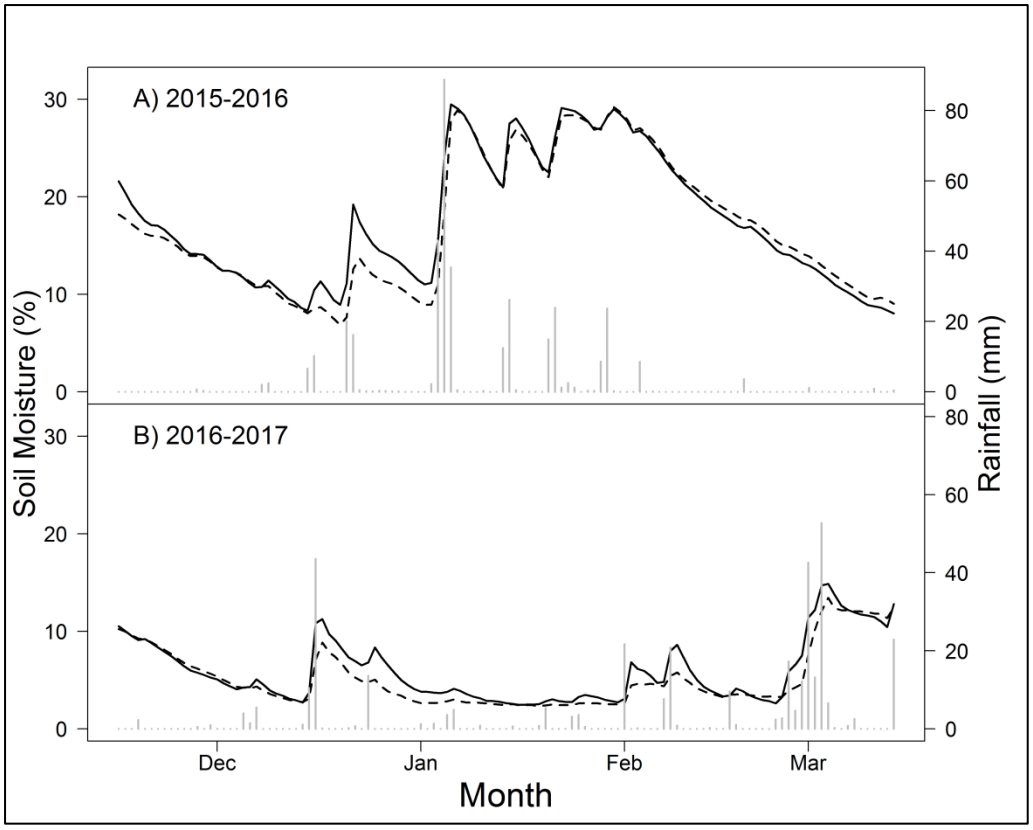


Figure 2.3. Mean daily soil moisture data for November 15th to March 15th for (A) 2015 to 2016 and (B) 2016 to 2017. *Solid* line indicates ambient plots and *dashed* line indicates sheltered/reduced rainfall plots. Grey vertical lines indicate rainfall events (right axes).

Soil total carbon (µg g^{-1}), nitrogen (µg g^{-1}) and phosphorus (µg g^{-1}) (**Table S2.2**) were quantified using four soil sub-samples per plot (0-15 cm depth) in both March 2016 and March 2017 using a soil auger (~10 cm dia). Samples were collected adjacent to strips harvested for aboveground biomass (detailed below). Samples were homogenized, sieved twice (2 mm, then 1 mm) to remove all roots/debris before being ground into a fine powder. Total soil C and N were assessed using an elemental analyser (2400 II CHN elemental analyser, Perkin-Elmer, USA) on samples collected in March 2016 and March 2017. Total soil P was analysed using an X-ray fluorescence (XRF) spectrometer (Epsilon 3^X, PANalytical). For XRF analysis, 1 g of soil was mixed with 50 mg boric acid before being made into a pellet using a base of 2 g of boric acid under 10 tonnes of pressure for ~60 seconds.

2.2.2 Aboveground Net Primary Production Data Collection

Live ANPP (LANPP) harvests were conducted in late February/early March to capture peak biomass in 2014, 2015, 2016 and 2017. Two 10 x 100 cm strips of plant material were removed at ground level (as per DroughtNet protocol). A different location was used each year to minimize the effects of repeated clipping. It is also worth noting that some carry-over green material could have been included in the samples of 'standing biomass'; however, it is highly unlikely given the nature of variable rainfall at this site (i.e. regular die-back of green material) and the 2-year turnaround on harvests locations. The two samples were pooled and subsampled (20-40% of total sample) for sorting by species. Samples were dried at 60 °C for 72 hours and weighed. Shannon's diversity (H) and equitability index (E_H) were calculated following Rosenzweig (1995). Simpson's diversity (*SDiv*) was calculated following Simpson (1949).

2.2.3 Plant Traits Data Collection

Plant functional trait data (see **Table 2.1** for list) were collected in February/March 2018 on those species that collectively contributed 95% of live ANPP (LANPP) for each plot from 2014 to 2018 (Garnier et al. 2004). Stem density was calculated in the field by counting the number of stems within a 10 cm^2 quadrat. Maximum vegetation height (to the tallest leaf base along the stem/tiller, in mm) was recorded. For all traits, when available, two individuals of each species were sampled per plot. Plants were subsequently excavated for leaf and root trait analysis. Excavation consisted of using a square-end shovel to cut 20 cm deep trenches around the entire individual then gently lifting the whole plug out of the ground. Only healthy, fully mature and unshaded individuals were selected for analysis.

Specific leaf area (SLA) was sampled on 5 leaves of each plant by scanning fresh leaves to calculate area, using WinFolia (WinFolia V 2015Pro, Regent Instruments Inc., Quebec, Canada). Specific leaf area was calculated by dividing area (mm^2) by dry mass (g). Leaf dry matter content was assessed by saturating 3 leaves in deionized water for a minimum of 6 hours (Garnier et al. 2001). Leaf dry matter content was calculated by dividing oven-dry mass (mg) by the water-saturated mass (g). Two dried leaves used for SLA were randomly selected and ground to a fine powder (~50 mg with 5 mg of tungsten oxide) for subsequent total C and total N analysis via combustion (TruSpec micro, LECO Corp, St. Joseph, MI USA; and FLASH EA 1112 Series CHN analyser, Thermo-Finnigan, Waltham, MA USA).

Roots were kept in loose soil at 4 °C until processed. Each root was gently shaken and rinsed to clean off excess soil. Roots were scanned using WinRhizo V 2013Pro (Regent Instruments Inc., Quebec, Canada) and were then dried (60 °C for 72 hours) and weighed. Specific root length was calculated as the ratio of total root length (cm) to dry mass (g). Root tissue density was calculated as the ratio of dry mass (mg) to total root volume (mm^3). Root

221 average diameter (mm) and number of root tips (#) were also assessed in WinRhizo. Root
222 branching intensity (RBI) was calculated as the ratio of root tips to total length (cm).
223
224 *2.2.4 Initial Data Analysis of Live Aboveground Net Primary Production*
225
226 Due to a gently sloping site topography there was a lack of separation in soil moisture across
227 treatments in the first two years of the study (**Figure 2.3**), particularly following periods of
228 high rainfall. We therefore conducted a preliminary analysis to determine the importance of
229 each of the treatments (shelter and P addition) on LANPP using linear mixed models (LMMs)
230 in R (V 3.2.5) with the '*lme4*' package. Marginal (fixed) and conditional (random) factor
231 adjusted R^2 values (R_M^2 and R_C^2, respectively) were calculated using the method developed by
232 Nakagawa and Schielzeth (2013) in the '*MuMin*' package in R. Maximum likelihood ratio
233 (ML) for linear mixed models (LMM) was used as recommended by Nakagawa and
234 Schielzeth (2013), although this is known to bias variance components (Bates 2010). Since
235 the goal was to attribute variance of dependent variables (i.e. 'Shelter', 'Phosphorus'
236 treatments and 'Year') we accepted this bias (i.e. variance estimates are calculated with both
237 marginal and conditional effects, rather than separately). A randomised, blocked design (one
238 replicate plot within each of four blocks) was used to account for site heterogeneity, with
239 'Plot' nested within 'Block' (both used as factors) for all analyses. Kenward-Roger Degrees
240 of Freedom Approximation was employed to estimate residual degrees of freedom for F-tests
241 in LMMs using the '*LMERTEST*' package (Kenward and Roger 1997; Halekoh and
242 Højsgaard 2014; Kuznetsova et al. 2015; Hasegawa et al. 2016). Post-hoc multiple
243 comparisons were achieved using Tukey 'single-step' comparison in the '*multcomp*' package
244 (Newell and Douglas 2014; Bretz et al. 2016; Hothorn et al. 2017) and used to determine
245 significance of fixed effects and their interactions. Homogeneity of variance was tested using

246 Levene's and Brown-Forsyth's tests (Box 1953; Markowski and Markowski 1990; Katz et al.
247 2009; Fox and Weisberg 2011; Crawley 2012).

248 Preliminary analysis indicated live ANPP increased each year from 2014 to 2016,
249 then declined in 2017 (**Figure 2.4**, $P < 0.001$), but Shelter and Phosphorus treatments (or
250 their interactions with year) had no significant effects ($P > 0.530$). Generally, greater annual
251 rainfall led to greater LANPP, with a high degree of within-site variation in LANPP in 2016.
252 The R_M^2 for the three-term (Year, Shelter and Phosphorus) LMM was 0.774, while the R_C^2 was
253 0.804. Year alone explained 75.2% of the variation in LANPP ($R_C^2 = 0.781$, $P < 0.001$).
254 Shelter ($P = 0.655$) and Phosphorus (P = 0.530) were both non-significant in the three-term
255 models. Shelter explained 10.8% ($R_C^2 = 0.108$, $P = 0.012$), while Phosphorus explained 11.3%
256 ($R_C^2 = 0.113$, $P = 0.013$) of the variation in LANPP. Therefore, we did not investigate the
257 treatment effects any further in this chapter.
258

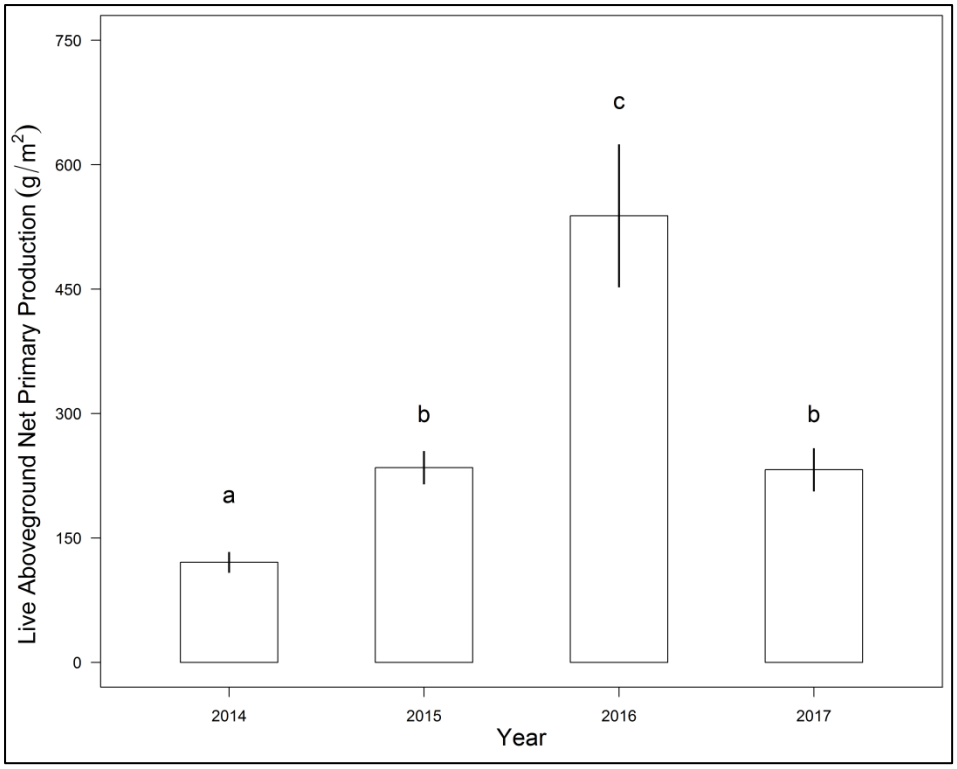


Figure 2.4. Live aboveground net primary production by year (mean ± standard error) harvested in late February/early March. Letters that are shared represent no significant difference (Tukey single step-wise comparison).

2.2.5 Preliminary Data Analysis for Plant Traits

Each trait was analysed using LMMs procedures (as described for the preliminary analysis of LANPP) to determine if 'Shelter or 'Phosphorus' treatments had significant effects on trait values within a species. If there were no effects, traits were pooled for subsequent analyses. Traits collected from plots in 2018 with significant treatment effects were excluded from analysis since there were no significant treatment effects on productivity leading up to the initial community in 2014, nor at any point through to 2017. We acknowledge that using traits collected in a single year may limit the strength of our conclusions given the potential importance of inter-annual variation in traits (e.g. Dwyer et al. 2014). However, it should be noted that other studies have used a single set of baseline trait values over a 25 year period, where the experimental treatments (grazing and N+P fertilisation) had no significant effects on trait values (Chollet et al. 2014).

2.2.6 Data Analyses for Diversity ~ ANPP Relationship

To determine the effects of initial community diversity or CWTs (for 2014) on LANPP in subsequent years (2015-2017), we used linear regression analysis in R (*lm* function). Eighteen CWTs (nine CWM and nine CWV), as well as three measures of taxonomic diversity (*H*, E_H, *SDiv*), from 2014 were examined for correlation with variability in LANPP (2015-2017). Live ANPP variation was calculated as the coefficient of variation within years for each plot. The LANPP CV was used to capture the breadth in variation in productivity of each plot under contrasting amounts of annual rainfall. In summary, we analysed plot-level estimates of diversity observed in 2014 to explain the variability in live biomass (**Figure S2.1**) in the subsequent three years. Data were log-transformed as needed and significant

correlations were considered at $\alpha = 0.05$. Shelter and Phosphorus treatments were not included, in order to attribute maximal variation (Adjusted R^2) to a single predictor (i.e. CWT). Other studies have utilised life-history traits (e.g. C3 vs. C4, annual vs. perennial, native vs. invasive) to investigate productivity patterns (McIntyre et al. 2006; Morgan et al. 2016; Stampfli et al. 2018). We chose to avoid life history traits because the dominant species used for trait analyses are all perennial, only one species is native, only one species is a non-grass and only one species has the C3 photosynthetic pathway (**Table S2.1**).

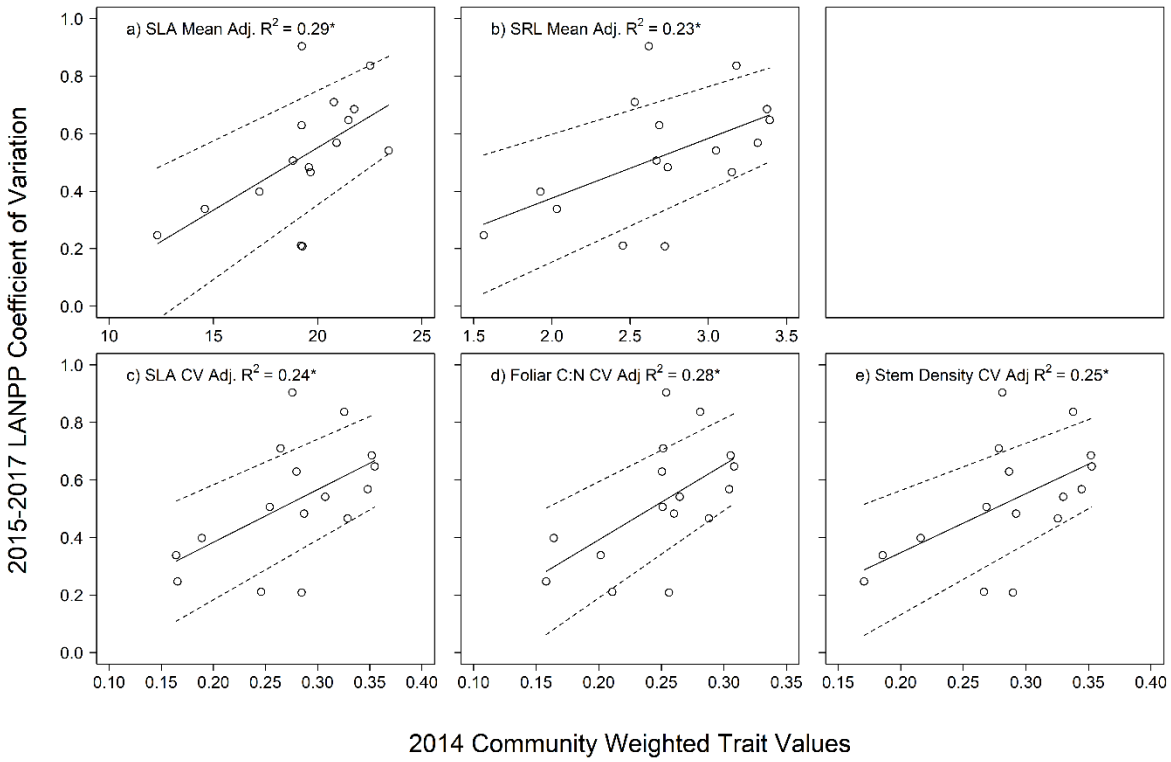
2.2.7 Data Analyses for Trait Coordination

The relationships between CWM, CWV and variation in LANPP were investigated via paired comparisons (i.e. all combinations tested against one another for correlation) using Pearson's correlation coefficient. In addition, traits for all species were pooled for correlation analysis in addition to each species individually. The assumption of linear relationships and normality was checked using scatterplots and Shapiro-Wilks test, respectively. The same analysis was conducted for traits of dominant species to investigate trait coordination. While a multiple linear regression method may be suitable to address such topics, as done by others (Lavorel et al. 2011; Chollet et al. 2014; Stampfli et al. 2018), the spatial and temporal replication in this study was relatively low and collinearity of predictors was unique for traits across species and CWTs (Graham 2003; Bretz et al. 2016), so multiple regression analysis was not carried out.

2.2.8 Data Analyses for Trait Frequency

Density plots (*density* function in R) were used to visualize inter- and intraspecific dispersion of traits. The *density* function in R disperses the observed data (in this case frequency of trait values) over a grid of a minimum of 512 points, and uses Fourier transformation to estimate

316 (using linear approximation) the kernel density. Density plots can provide useful insight into
317 niche-overlap and co-existence in biological communities (Mouillot et al. 2005).
318
319 ## 2.3 RESULTS
320
321 *2.3.1 Diversity ~ ANPP Relationship*
322
323 Diversity indices (*H*, H_E and *SDiv*) of the initial community (2014) did not significantly
324 explain the variation in productivity (LANPP CV) observed over the next three years (2015-
325 2017), which experienced high inter-annual rainfall variability. Of the nine CWM, only SLA
326 and SRL were positively correlated with LANPP CV (**Figure 2.5a-b**), while the associated
327 trait variances (CWV) for foliar C:N, SLA and stem density were positively correlated with
328 ANPP CV (**Figure 2.5c-e**).
329



330
331

332 **Figure 2.5.** Significant correlations between 2014 community-weighted mean (a-b) and
333 variance (c-e) traits with the coefficient of variation (CV) in live aboveground net primary
334 production (LANPP) from 2015-2017. Asterisks next to Adjusted R^2 values denote
335 significance where $P < 0.05$. Solid lines indicate regression fits and dotted lines indicate 95%
336 confidence intervals.

337

2.3.2 Trait Relatedness for the CWTs, the Community and Individual Species

As observed above (**Figure 2.5**), there were positive associations of LANPP CV with CWM of SLA, SRL and CWV of SLA, FolCN and StDen (**Figure 2.6**). There were positive relationships between CWM and CWV for most traits, with the exception StDen; however, for FolCN, LDMC and RTD these relationships were relatively weak, as denoted by the lighter blue coloration in **Figure 2.6**. Interestingly, Shannon's and Simpson's diversity indices were positively correlated with each other, but notably negatively associated with the CWM and CWV measures within this study.

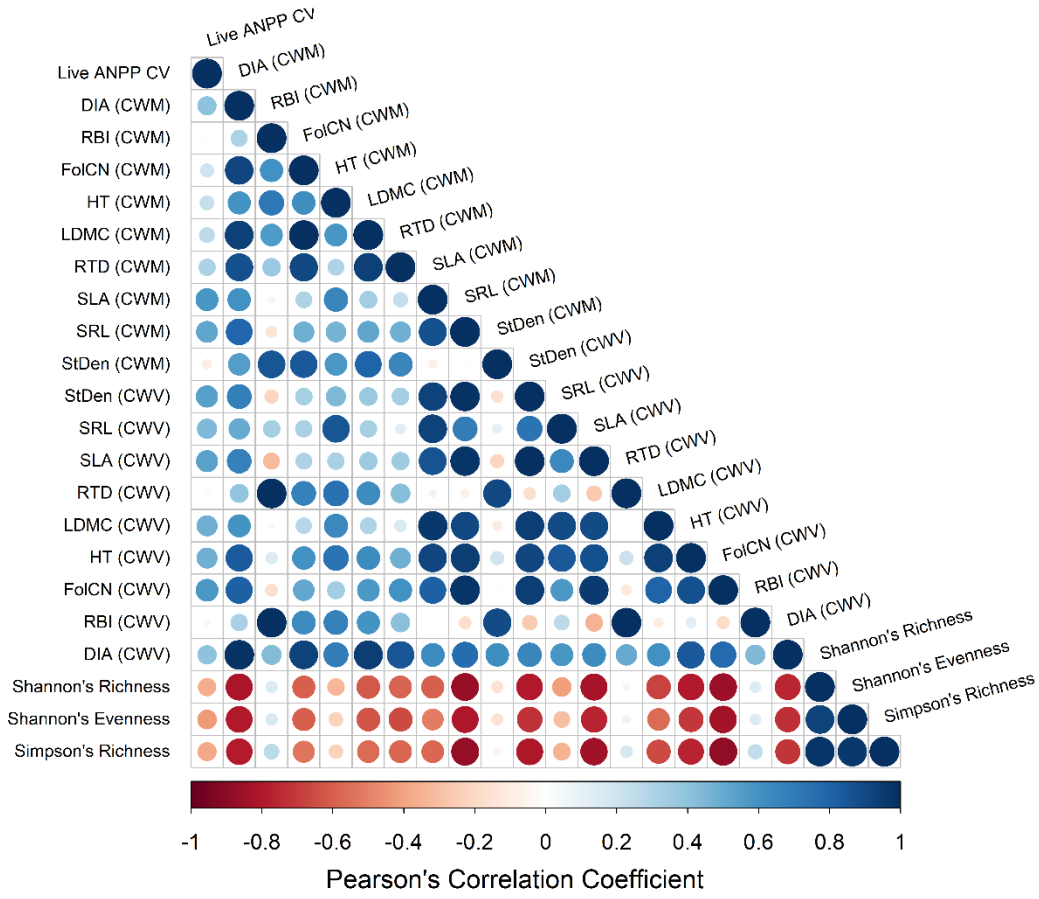
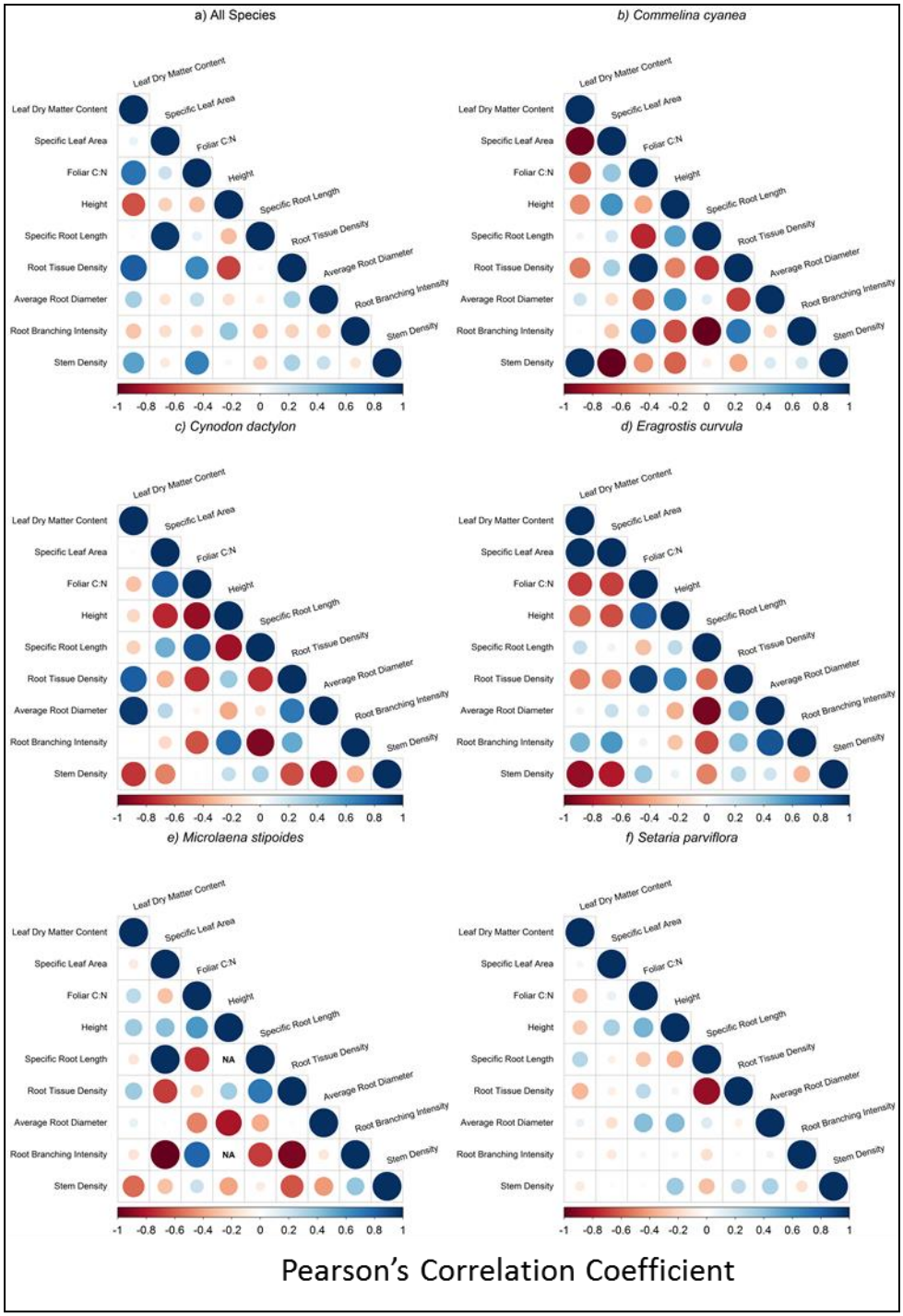


Figure 2.6. Pearson's correlation coefficients for all community-level measurements in this study including the coefficient of variation of live aboveground net primary productivity (LANPP CV), community-weighted mean and variance traits (CWM and CWV, respectively) and Shannon's/Simpson's diversity indices. DIA: mean root diameter; RBI: root branching intensity; FolCN: foliar C:N ratio; HT: plant height; LDMC: leaf dry matter content; RTD: root tissue density; SLA: specific leaf area; SRL: specific root length; StDen: stem density.

The correlations between traits within the individual species and combined (all species) datasets are examined in **Figure 2.7** (panels **a**, and **b**-**f**, respectively). Within the combined, (all species) community-level trait data there were strong positive correlations for FolCN-LDMC-RTD, SLA and SRL, and for StDen with FolCN-LDMC (but not with RTD) (**Figure 2.7a**). Strong negative relationships include HT and LDMC, and HT and RTD. Based on these results, we would assume HT would be negatively associated with FolCN (because of the strong positive correlations for FolCN-LDMC-RTD); however, it was only marginally so. On examining correlations for individual species, incredibly complex mixtures of correlation amongst trait were revealed, resulting in stark contrasts between species-specific and community comparisons, and species to species comparisons. For example, while the FolCN-LDMC-RTD correlation was observed for all species (combined) (**Figure 2.7a**), there was no evidence of such correlation in *C. cyanea* (**Figure 2.7b**), *E. curvula* (**Figure 2.7d**), *M. stipoides* (**Figure 2.7e**) or *S. parviflora* (**Figure 2.7f**). It was only observed in *C. dactylon* (**Figure 2.7c**), indicating that the strong positive trait relationships at the community-level is driven principally by *C. dactylon*. Similarly, the positive correlation of SRL and SLA observed across the combined species dataset was only apparent for *M. stipoides* (**Figure 2.7e**) in the single species analyses. Unlike the remaining species, *S. parviflora* appeared to have little correlation between traits (**Figure 2.7f**); however, RTD and SRL were strongly negatively correlated which was true for all other species except *M. stipoides*.



382

383 **Figure 2.7**. Pearson's correlation coefficients for plant functional traits across all species

384 combined (a) and each species individually (b-f).

385

386 *2.3.3 Trait Density and Coordination*
387
388 Density plots of each trait by species are presented in **Figure 2.8**. *Eragrostis curvula*
389 exhibited conservative aboveground traits (high LDMC, low SLA and high foliar C:N) when
390 compared to the remaining species (**Figure 2.8a-c**). With the exception of *C. dactylon*, the
391 remaining species were at the more acquisitive end of the spectrum for aboveground traits
392 (low LDMC, high SLA and low foliar C:N) (**Figure 2.8a-c**). *Cynodon* was unique in terms of
393 these three traits, in that it possessed intermediate LDMC with highly variable SLA and foliar
394 C:N values, suggesting it has the capacity to acclimate to environmental heterogeneity and
395 competition. Plant height (**Figure 2.8d**) and stem density (**Figure 2.8j**) were highly variable
396 for all species except *M. stipoides*. *Microlaena* is the only C3 grass sampled for traits in this
397 study, so it is likely a weak competitor (Grime 1988; Westoby 1998) during the hot summer
398 months, when strong growth/recruitment only occurs provided adequate rainfall (Chivers and
399 Aldous 2005). Aboveground traits indicate *E. curvula* is a slow-growing/conservative species
400 and *C. dactylon* is intermediate but flexible, while the remaining species were comparatively
401 acquisitive. On the other hand, belowground traits yielded unique species profiles.
402 Specific root length for *C. dactylon* was acquisitive (long, thin roots) while the
403 remaining species were on the conservative end of the spectrum (**Figure 2.8e**). *S. parviflora*
404 exhibited comparatively low values of RTD, suggesting it is on the more acquisitive end of
405 the resource spectrum. While the remaining species (*except C. cyanea*) exhibited more
406 conservative RTD values, only *C. dactylon*'s RTD was highly constrained (i.e. low
407 dispersion/CV of RTD) (**Figure 2.8f**). Like RTD, *C. dactylon*'s RBI was also highly
408 constrained and uniquely conservative relative to the remaining species (**Figure 2.8h** and **8i**).
409 Average root diameter was comparable across species (i.e. highly overlapping); however,
410 there appeared to be two diverging groups with *Microlaena* and *Setaria* having lower
411 diameters compared to *E. curvula* and *C. dactylon* (**Figure 2.8g**). Belowground traits depict

412 *S. parviflora* as a relatively acquisitive species. *Microlaena* was variable (acquisitive SRL
413 and RTD, conservative RBI and diameter). *E. curvula* was primarily conservative, with the
414 exception of having both high values and variability for RBI. A profile for *C. dactylon*
415 includes conservative, intermediate and acquisitive belowground traits; as with *C. dactylon*'s
416 aboveground traits, the variability in belowground traits suggests a species capable of filling
417 different niches.
418

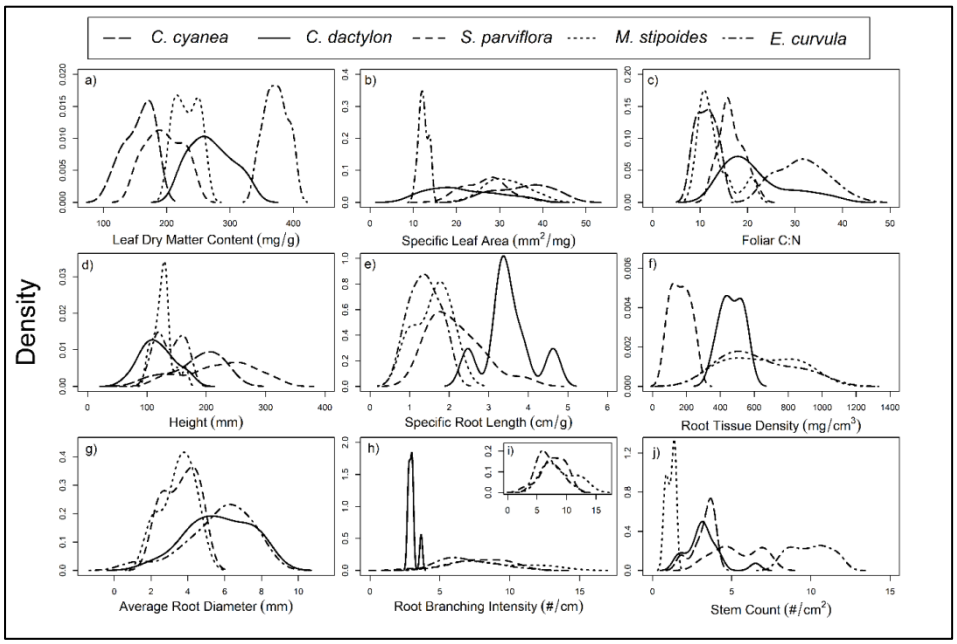


Figure 2.8. Trait density plots for nine traits of the five most abundant species. Note that *Commelina cyanea* is missing from belowground trait descriptions due to limited data. Inset in panel h has the x-axis rescaled to view data for *S. parviflora*, *M. stipoides* and *E. curvula*.

2.4 DISCUSSION

We hypothesized that the relative proportion of trait values associated with acquisitive and conservative resource use strategies would be useful in predicting rainfall-driven variability in productivity in our mesic grassland. Consistent with our hypothesis, we found that the abundance of species possessing trait values at the acquisitive-end of the resource-use continuum resulted in plots which had higher variability in productivity over three years. This appeared to be driven by low productivity of acquisitive species during drier years and higher production during wetter periods. Conversely, the abundance of species possessing conservative trait values resulted in stable growth during subsequent above- and below-average rainfall years. These results provide strong evidence that community weighted traits are useful predictors of ecosystem responses to altered rainfall regimes (Polley et al. 2013; Mouillot et al. 2013; Barkaoui et al. 2016). Such results suggest traits are useful in understanding how plant communities may respond to changes in rainfall predicted in the future.

In addition, we investigated whether CWM representative of an acquisitive plant community was associated with large intraspecific variation in trait (CWV). If so, while traditionally thought to be less stress tolerant, acquisitive species may have the capacity to withstand environmental perturbation as some individuals may not be affected (i.e. high trait dispersion results in less individuals' traits being filtered by the disturbance/stress). In some cases, we saw acquisitive CWM exhibit large variation (CWV), likely a result of acquisitive species being able to maintain the necessary flexibility to occupy niches regardless of its suitability. Conversely, if a species exhibited conservative trait values it is likely a superior stress tolerator and uses that strategy consistently (i.e. low variation) across the site. However, we

found that aboveground and belowground organs often exhibit different strategies associated with traits, thereby confounding classification of whole plants/species as conservative or acquisitive. While leaf and root strategy coordination has been observed (Liu et al. 2010), our study indicates this is not the case for these species. This is not surprising given that different resources are being targeted by the leaves (capturing C) aboveground and the roots (acquiring water and nutrients) belowground. Given predicted changes in climate, the need to manage diversity for ecosystem stability will remain crucial especially in the context of increasing human dependence on ecosystem services (e.g. clean water and air, carbon sequestration) (Lavorel et al. 2011; Lamarque et al. 2014). While species at all points on the acquisitive-conservative spectrum will contribute to productivity and associated ecosystem functions in the future, those at both ends of the spectrum will also contribute a unique aspect to stability (i.e. resistance and/or resilience) of their respective ecosystems (Grime et al. 1997; Reich 2014). In the future, targeting for traits related to particular management objectives will be key since these traits represent surrogates for life history strategy and are linked to ecosystem processes.

2.4.1 Plant Functional Traits, Strategy, and Community-Weighting

Research focusing on plant responses to stress and/or disturbance has been highlighted in recent years due to changes in climate. Physiological acclimation (plasticity) is the first determinant of whether or not an individual can survive a changing environment (Smith et al. 2009). Both physiological plasticity and natural intraspecific variation in traits related to fitness can change the underlying properties of plant communities, including their functioning and sensitivity to perturbation (Jung et al. 2010, 2014). The link between trait values and intraspecific variation has gained increasing attention as important components of ecosystem

stability and community assembly (Cianciaruso et al. 2009; Albert et al. 2010; De Bello et al. 2011; Albert et al. 2012). In our study, we found that both trait means and variances (CWM and CWV, respectively) are important predictors of stability in productivity, although the two metrics are not necessarily themselves correlated. For example, both the mean and variance of community-weighted SLA were significantly and positively correlated with variation in productivity, although they were not significantly correlated themselves. Conversely, foliar C:N mean and variance CWTs were related, but only the CWV was associated with stability in productivity. Furthermore, while SRL mean and variance were unrelated both were useful predictors of variation in productivity. These results highlight the importance of intraspecific variation in trait-based ecology, while suggesting inter- and intra-specific variability in traits may indicate unique aspects of ecological processes (Kichenin et al. 2013) and may further be separated between above- and below-ground processes (Ryser and Eek 2000; Laliberté 2017).

2.4.2 Plant Trait Dispersion and Coordination

The concept of an all-encompassing trait related to strategy or fitness remains elusive; however, suites of traits are typically linked to the "acquisition or conservation of resources" paradigm (Diaz et al. 2004). Therefore, it was unsurprising that traits related to resource acquisition (SLA, SRL, foliar C:N) were related to ANPP CV. While coordination of these traits was observed in some species for aboveground traits (e.g. *E. curvula* had comparably conservative leaf traits), this was not necessarily observed for belowground traits. For example, *C. dactylon* exhibited high aboveground trait variation, but had low variation (i.e. conservative) in belowground traits. In *S. parviflora*, aboveground and belowground traits reflect acquisitive strategies, while it was not possible to consistently attribute these traits to a single strategy in *Microlaena*. Hence, the conservative-acquisitive paradigm may not be

consistently observed for above- and belowground traits when there is not uniform coordination of processes and function within a single plant species (Schroeder-Georgi et al. 2016). This highlights the need to further explore traits across multiple species in mixed plant communities, to increase understanding of their role in ecosystem stability and functioning.

2.4.3 Plant Functional Traits and Ecological Theory

In this study, we found that the community composition (i.e. means and variances) of traits related to growth and morphological strategies were important predictors of ANPP stability. As suggested by the "biomass-ratio hypothesis" the dominant species (and associated traits and strategies) were the best indicators of stability in primary production. In addition, the separation of species-specific (or interspecific divergence) of trait values was evident in both above- and belowground traits suggesting that hypotheses such as "Walter's two-layer" and the "pulse-reserve" paradigm may be applicable to mesic grasslands where neighbouring species exhibit contrasting traits (i.e. minimal overlap of trait values). While such hypotheses have been typically applied to understanding extreme divergence of traits in arid ecosystems (e.g. deserts and savannas), these concepts were also found to be relevant in this study. These arid ecosystem-based hypotheses, alongside the trait-based approach, provided a potential mechanistic explanation for the rainfall-productivity relationship observed in our mesic grassland and may be applicable to grasslands worldwide. As highlighted in the work of Moles (2018), increasing data availability and technology to measure plant functional processes will be useful in addressing climate impacts on diversity and ecosystem function. However, empirical testing and development of ecological theory will be crucial to place these data into a context suitable for broader audiences and the international scientific community.

Overall, we found strong differences amongst our dominant grassland species with respect to PFT responses to reduced rainfall and P addition. While many traits were unaffected by drought or P addition, we observed contrasting directional responses of PFTs across species. For example, SRL increased with P addition in *Microlaena*, but decreased in *Cynodon*; this is not unexpected since differences in grass root response to P addition are often species-specific (Fort et al. 2014). While we attribute these changes to the direct effects of P addition, we did not investigate the indirect effects of P addition which could potentially alter PFT responses (e.g. intransitive dynamics or complementarity, Soliveres et al. 2015). For example, with limited niche space for root exploitation, a direct effect of P addition on SRL of *Cynodon* could result in a shift in root traits of *Microlaena,* via competition. Alternatively, potential unique P requirements between *Microlaena* and *Cynodon* could result in opposite responses to fertilization (Lambers et al. 2006). Others have observed P uptake to be directly related to root traits (namely specific root hair cylinder volume) which has consequences for productivity responses to fertilization in both grass and legumes (Haling et al. 2016). These results highlight the complexity of trait-environmental relationships in (semi-)natural plant communities and warrants further study, ideally through the use of coordinated experiments to determine how site-specific characteristics may influence PFTs underpinning drought and fertilization responses (Fraser et al. 2013; Ploughe et al. 2018)

2.5 CONCLUSIONS

While productivity in grasslands as a function of rainfall (e.g. historic mean annual, seasonal, variation) has been well-studied, a mechanistic relationship between plant diversity and ecosystem stability remains elusive. Here, we found evidence that traits provide a mechanistic link between plant composition and stability in productivity, especially those traits related to resource acquisition and storage. The relative dominance of acquisitive or

554 conservative plant species (and associated traits) will contribute substantially to grassland
555 response to future changes in rainfall. A high abundance of acquisitive species may lead to
556 greater productivity during periods with adequate or above-average rainfall, but will
557 contribute to losses in productivity during drought periods. Conversely, species with
558 conservative strategies and associated traits are likely to maintain ANPP during below-
559 average rainfall, but not substantially increase ANPP during above-average precipitation. We
560 conclude that intraspecific variation in traits remains a crucial aspect of trait-based ecology
561 and we found evidence to suggest it may be related to plant resource-use strategy, and
562 therefore predictive of ecosystem response to environmental variation in the future.
563

Chapter 3: Root functional traits are key drivers of grassland response to drought and P fertilization

In review with *Plant and Soil*

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AUTHOR CONTRIBUTIONS: JC conceived the ideas, acquired data, analysed data and wrote the manuscript. UN, DT and SP designed the experiment and contributed critically to manuscript development.

3.0 ABSTRACT

Rainfall patterns and soil nutrients play an important role in regulating grassland productivity worldwide. We examined the effects of prolonged, severe drought (65% reduction in rainfall) and phosphorus fertilization on aboveground net primary production (ANPP) and plant functional traits of four dominant mesic grassland species (*Cynodon dactylon*, *Microlaena stipoides*, *Eragrostis curvula* and *Setaria parviflora*, collectively representing ~93% of total aboveground net primary productivity (ANPP) at our site) to identify mechanisms underpinning species specific responses. In addition, we measured stomatal conductance following a series of rainfall events to understand how changes in traits potentially relate to physiological responses. Reduced rainfall decreased ANPP by 29 % while P addition increased ANPP by 62 %; however, there was no interaction between P addition and drought treatments for plant ANPP, indicating P addition did not alleviate the effects of drought. At the species-level, only *S. parviflora* exhibited a decrease in ANPP under drought, while *C. dactylon* was the only species to respond significantly to P addition. Root tissue density increased and specific root length decreased in *C. dactylon* under P addition and both changes in traits were associated with greater ANPP. Foliar C:N, leaf dry matter content and root

diameter were unaffected by rainfall or phosphorus treatments in any of the species sampled. Stomatal conductance, however, was affected by the interaction of drought and phosphorus, but only periodically and predominately in *C. dactylon*. Principal components analysis indicated that root tissue density is a key trait in this system, and overall was the principal trait associated with the recovery of plant physiological function following late summer rainfall events. Our results indicate that this mesic grassland was sensitive to rainfall events, regardless of their magnitude. Belowground traits are key determinants of species responses to water availability and while these traits can be modified by P addition, P had little direct effect on ANPP and did not modify the effects of drought in our study.

3.1 INTRODUCTION

Grasslands cover 29.3% of ice-free terrestrial land-area worldwide (Tanentzap and Coomes 2012; Hooke et al. 2012) and hold greater than one-third of the terrestrial carbon stock (Trumper 2009). Plant productivity and associated ecosystem processes in grasslands are primarily driven by rainfall patterns, with soil nutrient availability contributing to differences among sites with similar climates (Suttie et al. 2005; Guo et al. 2012; Fay et al. 2015). Aboveground net primary production (ANPP) is strongly related to carbon sequestration (Scurlock and Hall 1998; Scurlock et al. 2002), but the ability of grasslands to sequester C is likely to be impacted by climate change. Global climate models predict reductions in the frequency of rainfall events resulting in fewer events that are greater in magnitude (Easterling et al. 2000; Seneviratne et al. 2012; IPCC 2013), a prediction that is likely to have negative impacts on the structure and function of grassland ecosystems (Knapp et al. 2001; Knapp et al. 2002; Fay et al. 2011; Walter et al. 2012). The absolute size of rainfall-related reductions in plant productivity will depend on soil fertility and will vary with management practices

such as fertilization. For example, Hofer et al. (2017) observed that differing soil nitrogen (N) contents affected productivity responses of grasses and legumes following a severe drought.

The link between ANPP and rainfall is well established for terrestrial ecosystems (Huxman et al. 2004). Reduced water availability typically leads to down-regulation of photosynthesis resulting in lower biomass accumulation at the individual and community level (Medrano et al. 2002; Ripley et al. 2010). While this relationship has been widely studied, a framework for how and why communities vary in their sensitivity of ANPP to changes in rainfall remains elusive. In southeast Australia, predicted changes in rainfall include fewer days of rain, more days with heavy rain, and a greater contribution of those heavy rain events to the total rainfall received (Evans et al. 2017). These changes will generate longer dry periods followed by short-lived wet periods, similar to the pulse-dynamics experienced in more arid ecosystems (Austin et al. 2004).

Sensitivity of grassland ANPP to disturbances is likely mediated by the life history strategies and associated plant functional traits (*sensu* Reich 2014), of the species found within those communities. For example, species that grow rapidly typically possess low tissue densities (i.e. high volume or area to mass ratios), a result of limited C investment into long-lived structural components. Species with high density tissues, associated with longevity and resistance to biotic and abiotic stressors, typically undergo slow growth. This spectrum of plant form and function has been described as the "resource-use" axis (Grime et al. 1997) and represents the inevitable trade-off between the ability of a plant to rapidly acquire resources and the increasing rate of tissue loss/turnover. Rapidly growing species are considered acquisitive, while slower growing plants can be considered as conservative. The relative abundance of acquisitive and conservative strategies in a community will lead to either resistance (i.e. ability to withstand disturbance, such as drought) or resilience (i.e. ability to recover following disturbance) of ANPP in grassland communities (Tardif et al. 2014).

Important traits associated with drought resistance include traits that allow the maintenance of growth during declining soil moisture levels, such as the possession of dense tissues for water retention to maintain photosynthesis or a high capacity for soil water uptake (i.e. high root mass fraction or high root length) (Volaire et al. 2014). Important traits associates with drought resilience include those that support rapid re-growth when soil water becomes available again, such as high leaf area to leaf mass ratio. Therefore, the sensitivity of ANPP in grasslands to drought will depend on both the abundance of traits related to acquisitive/conservative strategies to cope with drought, and the change in rainfall patterns relative to previous regimes.

Changes in root characteristics as a result of long-term fertilization in grasslands are likely to alter plant responses to drought. For example, there is evidence that increased soil P content increases root diameter and lowers length/mass ratios (i.e. less surface area and foraging potential) (see Lambers et al. 2006 for review), which could negatively affect soil water absorption. In addition, high soil P availability may limit mycorrhizal colonization and thus negatively affect water uptake (Hetrick et al. 1990), although this relationship is likely not universal across plant and fungal taxa (Osonubi 1994; Ruiz-Lozano et al. 1995; Augé 2001). dos Santos et al. (2006) observed enhanced photosynthetic recovery following a mild water deficit of *Phaseolus vulgaris* L. when fertilized with P. Such morphological or physiological changes in grassland plant species as a result of P addition are likely to have consequences for ecosystem resistance and resilience to drought. There is also the potential for changes in soil nutrient availability to drive shifts across the acquisitive-conservative resource-use axis at the community scale that may moderate system-level drought impacts.

The overall objective of this study was to investigate the response of a mesic grassland community that had undergone P fertilisation (no P and 10 g m^{-2} y^{-1} for four years) and reduced rainfall (65% reduction from ambient rainfall) to a series of rainfall inputs

following a prolonged inter-pulse period (Huxman et al. 2008; Shriver 2017) that had resulted in nearly total foliar dieback. Rainfall inputs were applied artificially and occurred in tandem with natural events over forty days, with half the site receiving only 35% of those amounts (i.e. 65% reduction in rainfall) reflecting the long-term drought treatment. We used traits and repeated measures of stomatal conductance to understand potential mechanisms underlying species-specific responses. Specific questions we addressed included: 1) does soil P addition alleviate the negative effects of reduced rainfall on community-level ANPP through changes in species-specific ANPP?; 2) do dominant plant species within a community respond similarly to reduced water availability and P addition, in terms of ANPP and traits?; and 3) how are the traits underpinning ANPP affected by reduced water availability and P addition? Additionally, we asked (4) do species-specific changes in traits from drought and P addition correlate with changes in species-specific productivity and (5) what is the extent of trait coordination within this community?

Providing a trait-based framework for these questions will help identify options for managing traits in grassland ecosystems to minimize the potential negative effects of anticipated future rainfall regimes. In addition, understanding how nutrient management and climate may affect grassland productivity will assist in developing more mechanistic and accurate predictions of terrestrial feedbacks on the global carbon cycle.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Site and Design

The field site was located at Western Sydney University's Hawkesbury campus (-33.613586, 150.738047). Mean annual precipitation (1913-2013) is 807 mm (Australian Government Bureau of Meteorology, Richmond – UWS Hawkesbury Station, Station Number 67021) and

summer temperatures range between 18.8 and 29.4 °C, while winter temperatures range between 3.2 to 17.3 °C. The site was grazed by cattle for ~40 years until it was fenced in 2013 to exclude large herbivores. The soil (90.2 ± 1.3% sand, 5.6 ± 1.4% clay) is Blackendon Sand with a hardpan at ~70 cm depth across the site. The most abundant species at the site are *Cynodon dactylon* (L.) Pers., *Setaria parviflora* (Poir.) Kerguélen, *Microlaena stipoides* (Labill.) R.Br. and *Eragrostis curvula* (Schrad.) Nees, which accounted for 61.4%, 17.3%, 11.8% and 2.8% of the plant community live ANPP, respectively, averaged from 2014 to 2018 (total live ANPP contribution of 93.3% for all four species). All species are perennial C4 grasses, except *M. stipoides* which is a C3 grass (Field and Forde 1990; Johnson et al. 2014). The next most abundant species, the C3 forb *Commelina cyanea* R.Br. (Thorpe 1980), accounted for only 1.7% of live ANPP . In addition to these five species, a further 21 species were recorded – all representing <1% of the plot-level biomass – at the site during this 4 year period.

Sixteen plots (25 m^2 and a minimum 2 m apart) were established in 2014. Four plots were fertilized in June 2014 at a rate of 10 g m^{-2} y^{-1} total P equivalent using Triple Superphosphate [P_2O_5] (Ameropa Australia, Melbourne VIC 3004) as described in the NutNet protocol (Nutrient Network, http://www.nutnet.umn.edu/). In June 2015, eight plots had rainfall exclusion shelters installed and four of those plots were fertilized (first fertilization in 2015) as above. Rainfall shelters (3 x 3 m) used clear acrylic plastic (Perspex) to exclude 65% of ambient rainfall, a level reflecting the lowest annual rainfall in the last 100 years (268 mm in 1944). A 1-in-100 year drought was chosen as the rainfall treatment as the field site is part of the international Drought Network (DroughtNet, https://wp.natsci.colostate.edu/droughtnet/). The two fertilization treatments ("no P" and "P+") and two rainfall treatments ("ambient" and 65% reduced or "sheltered") were crossed in a factorial design across four blocks with one replicate per block.

716 Interception of photosynthetic photon flux density (PPFD) was recorded at 15 minute
717 intervals (Apogee sensors, model SQ-110, ICT International, Armidale, NSW, Australia) in
718 one ambient and one sheltered plot; shelters intercepted < 18% PAR). Soil moisture content
719 was recorded at 5-minute intervals starting November 11th 2015 using time-domain
720 reflectometry (TDR) sensors (CS606, Campbell Scientific, Thuringowa, QLD, Australia).
721 One TDR sensor was used in each plot and recorded the soil moisture content of the top 15
722 cm of soil Soil moisture sensors were checked periodically for accuracy using a handheld soil
723 theta probe (Delta T Devices, UK). Rainfall data were obtained from the nearby EucFACE
724 site (Ellsworth et al. 2017). From April 24th to October 5th 2016, rainfall gauges were
725 installed under two shelters and in one ambient plot to test the effectiveness of shelter rainfall
726 interception (target was 65%). Over that time period, 347 mm rain was recorded in the
727 ambient plot while the sheltered plots recorded 148 mm (i.e. 57.5 ± 5.4% interception).
728
729 *3.2.2 Watering Regime*
730
731 From December 10th 2017 to February 8th 2018 (summer in the Southern Hemisphere), the
732 site received 55 mm precipitation, with 4 mm in the last 15 days. Potential evaporation (mm)
733 was 403.1 (bom.gov: RAAF, station # 067105) over that time and mean site soil moisture
734 content was 2.7 ± 0.2% as of early February. These conditions were uncharacteristically low
735 for this site and thus vegetation was uniformly drought stressed across the site. For reference,
736 over a similar time period (December 10 to February 8) from 2015 to 2017 the site received
737 219, 350 and 146 mm, respectively. Following this long, dry (inter-pulse) period, plots were
738 manually watered to investigate recovery of physiological function of the four dominant plant
739 species under the two rainfall regimes and two P regimes. Rainfall and soil moisture data
740 from the previous years indicated that two 20 mm events (~1 week apart) would raise soil
741 water content in ambient plots to levels required to elicit a productivity response. The first 20

742 mm event was applied to ambient plots on February 8th and the second on February 13th 2018
743 (**Figure 3.1**). Sheltered (drought treatment) plots received 65% less than the ambient plots
744 (i.e. 7 mm per event). Water was applied using a tank-and-pump system, and amounts were
745 monitored using flow-meters.
746

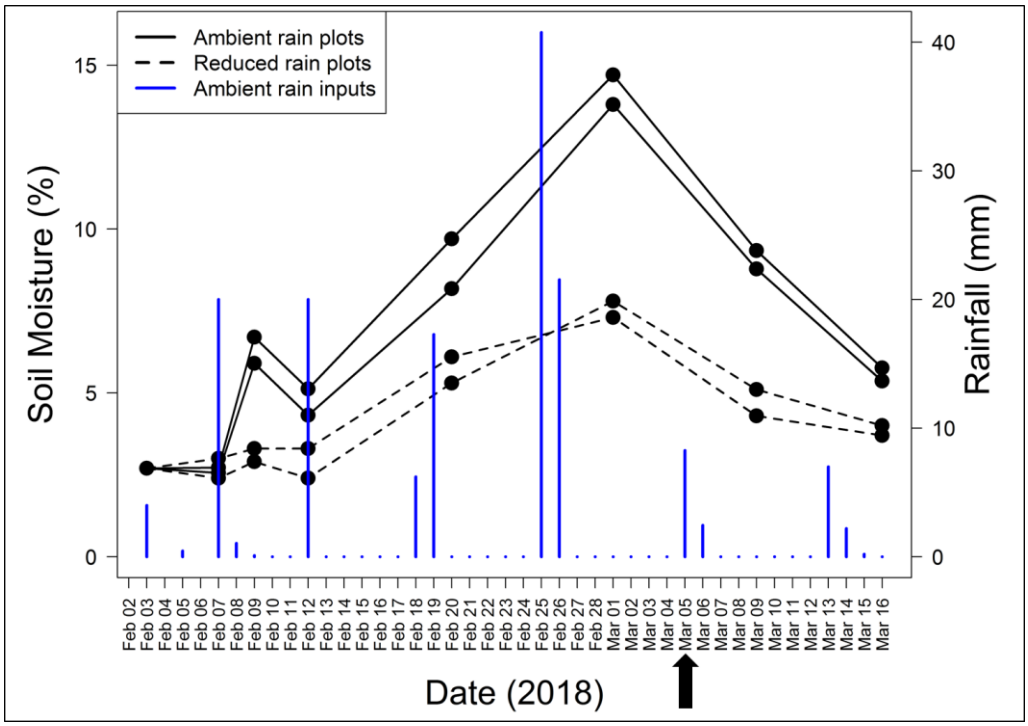


Figure 3.1. Soil moisture content for ambient rainfall (solid black lines, dots indicate day of sampling) and reduced rainfall (dashed lines, dots indicate day of sampling) plots. Blue vertical lines indicate ambient rainfall inputs including two experimentally applied 20 mm events (February 7th and 12th). Reduced rain plots received 35% of ambient rainfall amounts (i.e. 65% reduction). Aboveground net primary production harvests occurred on March 5th 2018 (denoted with black arrow). Plant functional trait data were collected March 5th -16th 2018 on new growth.

3.2.3 Plant Productivity

Aboveground NPP data were collected in late March 5th 2018, to capture peak biomass (Chieppa *Chapter 2*). Prior to rainfall inputs in February 2018, the site had primarily standing dead material from the dry inter-pulse period so the collected green material represents nearly all growth which occurred as a result of the irrigation inputs. In each plot, two 10 x 100 cm rectangles (as per DroughtNet protocol) were clipped at ground level on 5 March 2018 to determine live ANPP (following re-watering). The two samples were pooled, mixed and subsampled (30% of total sample) for sorting by species (dead material removed). Samples were dried at 60 °C for 72 hours and weighed for dry mass.

3.2.4 Stomatal Conductance and Leaf Chlorophyll Content

During each day of sampling (**Figure 3.1**) three individuals of each species were sampled for stomatal conductance (g_s, $mmol \cdot m^2 \cdot s$) using a portable leaf porometer (Decagon Devices Inc., Washing, USA). Measurement days were chosen for minimal wind and cloud cover to obtain the highest g_s values for three individuals of the four dominant species from one block, per treatment. Previous sampling campaigns indicated best sampling times were between 12:00 to 16:00 (i.e. full sun, highest vapour pressure deficit period for that time of year). Soil moisture of each plot was taken in five locations within the plot using a handheld TDR, and averaged (**Figure 3.1**). In summary, measurements of g_s and soil moisture were taken on the 7th, 9th, 12th and 20th of February and the 1st, 9th and 16th of March. Initial soil moisture readings were taken at the commencement of the sampling period on February 3rd.

3.2.5 Plant Functional Trait Collection

Functional trait data were collected from 5th - 16th March 2018 on new growth that followed the re-watering events, on unshaded, healthy individuals. Trait data were collected for the four most dominant species (mean live ANPP contribution of 93.3% from 2013 to 2018). Two individuals of each species were selected in each plot and measured for maximum height using a meter stick (Cornelissen et al. 2003). For each individual, two leaves were collected and placed in plastic vials, filled with deionized water and rehydrated for a minimum period of 6 hours (Garnier et al. 2001). Water-saturated leaves were surface-dried, weighed and scanned using WinFolia (V 2015Pro, Regent Instruments Inc., Quebec, Canada) for leaf area determination. Leaves were subsequently dried at 60 °C for 72 hours and weighed for dry mass. Leaf dry matter content was calculated as the ratio of dry mass to saturated mass (LDMC, mg g^{-1}). Specific leaf area was calculated as the ratio of saturated leaf area to dry mass (SLA, mm^2 mg^{-1}). Dried foliage was ground to a fine powder and measured for total C and N content using an elemental analyzer (2400 II CHN elemental analyzer, Perkin-Elmer, USA). Foliar C/N ratio was calculated as the ratio of total C content (%) to total N content (%) (FolCN, unitless).

Individuals were flagged to ensure above- and below-ground traits were collected on the same plants. Whole plants were carefully excavated (~25 cm depth) and stored in plastic bags in a cooler. Roots were kept at 4 °C until analysis. For analysis, roots were gently washed over a 1-mm sieve before being scanned using WinRhizo (V 2013P, Regent Instruments Inc., Quebec, Canada). After scanning, roots were dried at 60 °C for 72 hours and weighed. Specific root length was calculated as the ratio of total root length to dry mass (SRL, cm g_{-1}). Root tissue density was calculated as the ratio of total root volume to dry mass (RTD, mm^3 mg^{-1}). Root diameter was considered the mean diameter for the entire root image (DIA, mm). Root branching intensity was calculated as the ratio of total root tips by total root length (RBI, # cm^{-1}).

808

809 *3.2.6 Data Analysis*

810

811 All analyses were conducted in Rstudio (V1.0.143, R V3.2.5). Data were log transformed
812 when necessary to ensure homogeneity of variance which was tested with Levene's and
813 Brown Forsyths tests (Katz et al. 2009). The effects of 'shelter', 'phosphorus', and their
814 interaction on total ANPP (i.e. all species), species-specific ANPP, and species-specific traits
815 were tested using linear mixed models (*lme4* package). 'Block' was included as a random
816 effect while 'shelter' and 'phosphorus' were fixed. Residual degrees of freedom were
817 estimated using Kenward-Roger Degrees of Freedom Approximation (*LMERTEST* package)
818 (Kenward and Roger 1997). Post-hoc Tukey 'single-step' comparison was employed when
819 fixed effects were significant (*multcomp* package) (Newell and Douglas 2014; Bretz et al.
820 2016; Hothorn et al. 2017). The same analyses were completed for each of the eight PF traits
821 Ts included in this study. To further investigate the relationships between traits, we
822 conducted principle components analysis (PCA) to determine relative importance and
823 interrelatedness of morpho-physiological characteristics (i.e. traits). Principle components
824 analysis was conducting using the *vegan* package. Data were transformed using the
825 'Hellinger' method (*decostand* function) and variance was scaled (Oksanen et al. 2007).

826

827 **3.3 RESULTS**

828

829 *3.3.1 Aboveground Net Primary Production*

830

831 Reduced watering amount resulted in reduced ANPP compared to ambient plots (29%
832 decline, $P = 0.018$) while plots which received Phosphorus had increased ANPP compared to
833 those that did not (62% increase, $P = 0.003$) but there was no interaction between these
834 treatments ($P = 0.103$, **Table S3.1** and **Figure 3. 2**). At the species level, ANPP for *Setaria*

835 decreased under reduced rainfall (P = 0.004), while *Cynodon* ANPP increased with P
836 fertilisation (P = 0.003, **Figure 3.2**). *Microlaena* ANPP was not significantly affected by
837 Rainfall or Phosphorus alone. There was, however, a significant two-way interaction for
838 *Microlaena* ANPP (P = 0.036), although post-hoc analysis indicated that treatment means
839 were not significantly different (P = 0.074).
840

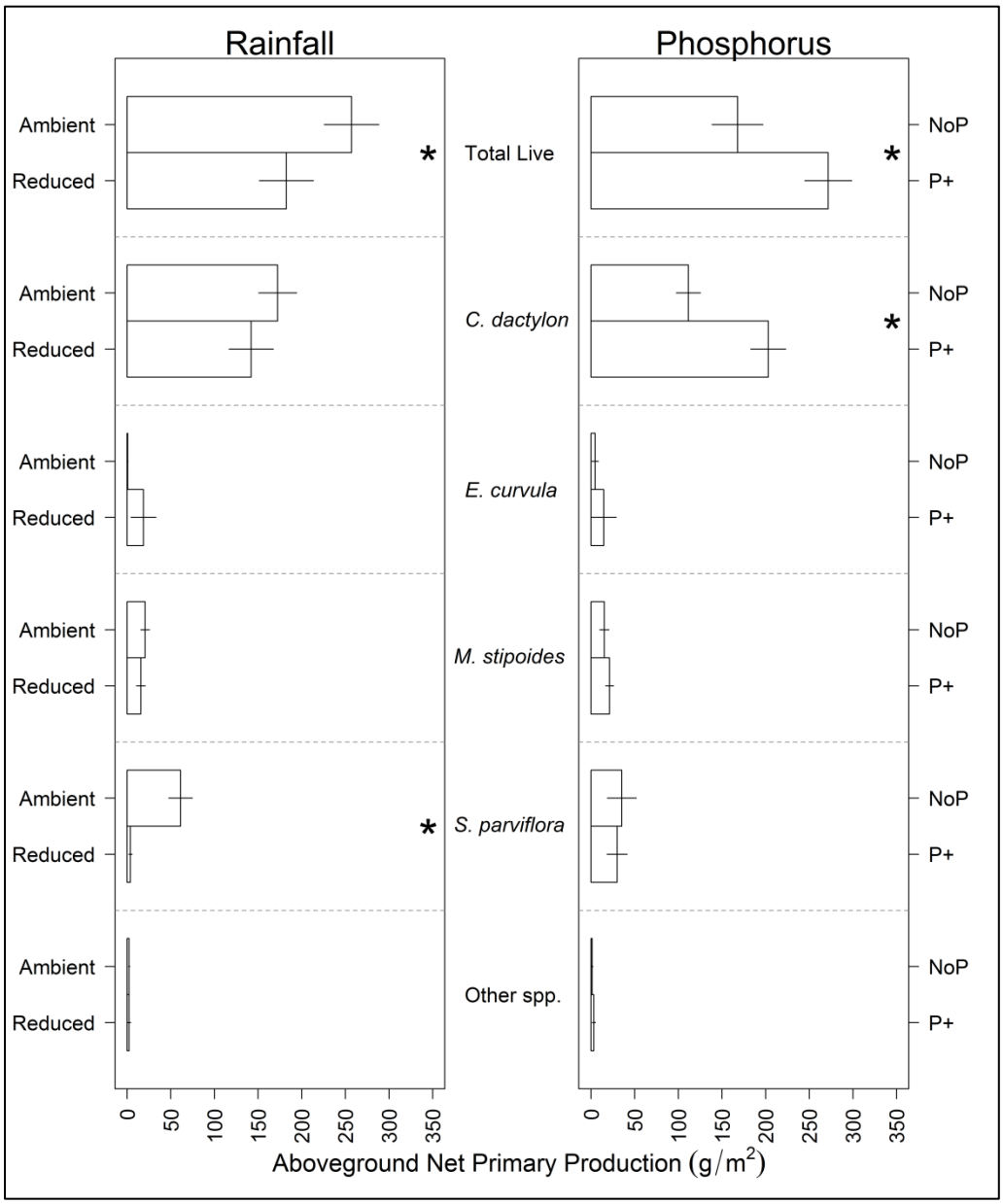
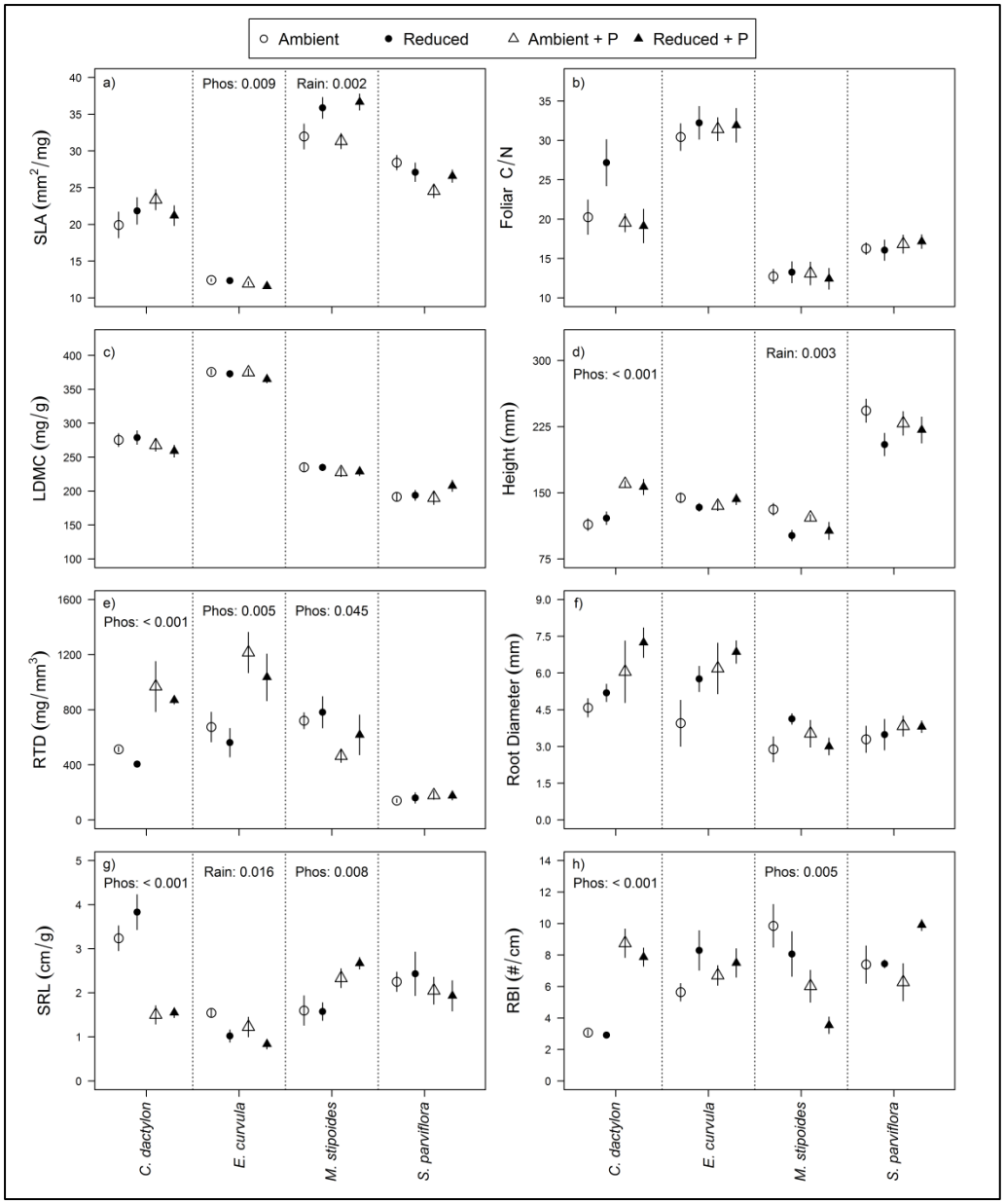


Figure 3.2. Live aboveground net primary production by rainfall (left panel) and phosphorus (right panel) treatments, separated for: all species (Total Live), dominant species (*Cynodon dactylon*, *Eragrostis curvula*, *Microlaena stipoides*, *Setaria parviflora*) and non-dominant species (Other spp.). Plots with asterisks represent significant differences at $P < 0.05$.

3.3.2 Species' functional trait responses to drought and phosphorus

Specific leaf area decreased significantly in *E. curvula* with P fertilisation; however, the change appeared minor with respect to interspecific differences in SLA (**Figure 3.3a**). In *M. stipoides*, SLA increased under reduced rainfall (**Figure 3.3a**). Foliar C/N and LDMC were unaffected across treatments for all species (**Figure 3.3b** and **3.3c**, respectively). Height increased with P addition for *C. dactylon* and decreased for *M. stipoides* under reduced rainfall (**Figure 3.3d**).

In terms of belowground traits, phosphorus addition led to significant increases in RTD for *C. dactylon* and *E. curvula*, although it had the opposite effect in *M. stipoides* (**Figure 3.3e**). Phosphorus addition had opposing effects on SRL in *C. dactylon* (decreased with P addition) and *M. stipoides* (increased with P addition) (**Figure 3.3g**). Reduced rainfall resulted in decreased SRL in *E. curvula*. Like RTD, P addition had contrasting effects on RBI between species, with *C. dactylon* increasing and *M. stipoides* decreasing values of this trait under added P (**Figure 3.3h**). Neither P addition nor reduced rainfall, or their interaction, had a significant effect on any traits measured for *S. parviflora* (**Figure 3.3**).



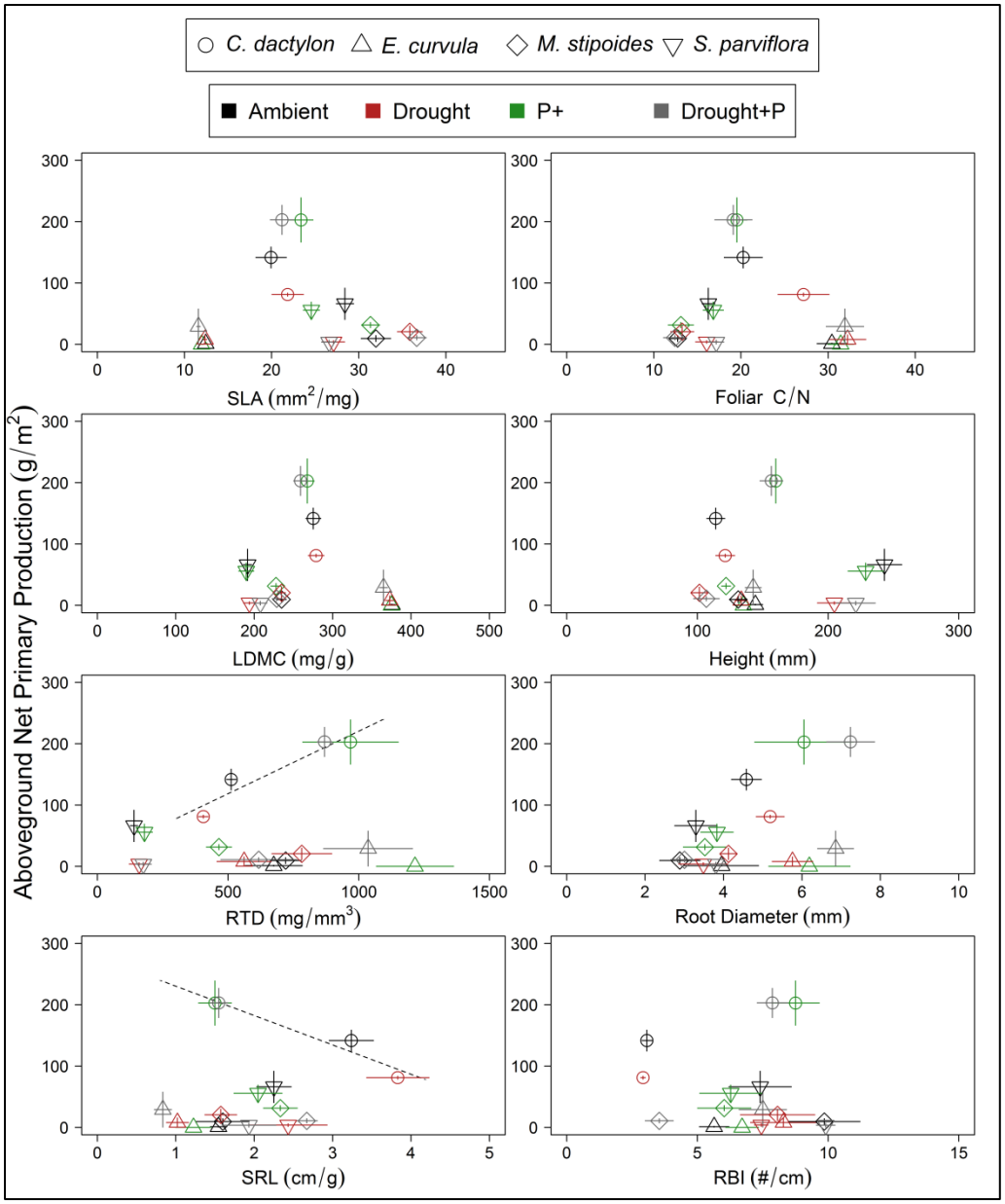
865

866 **Figure 3.3**. Plant functional traits in different treatments for the four dominant species.
867 Abbreviations labelling y-axes represent (panel a) specific leaf area, (c) leaf dry matter
868 content, (e) root tissue density, (g) specific root length, and (h) root branching intensity.
869 "Phos" denotes the *P*-value resulting from linear mixed models (LMMs) for the phosphorus
870 treatment. "Rain" denotes the *P*-value resulting from LMMs for the rainfall treatment. *P*-
871 values below 0.05 are considered significant; however, *P*-values between 0.05 and 0.10 are
872 also reported for clarity.

873

3.3.3 Plant functional traits underlying changes in ANPP

Only two traits were significantly related to ANPP, and this was solely for *C. dactylon*. Hence, intraspecific changes in traits as a result of treatments for the remaining species (**Figure 3.3**) did not result in changes in ANPP. For *C. dactylon*, increased RTD as a result of P addition was associated with greater ANPP (R^2 = 0.86, *P* = 0.048, **Figure 3.4**), likely underpinning the ANPP response observed across treatments (**Figure 3.2**). Similarly, SRL decreased with P addition, which was associated with lower ANPP than *C. dactylon* in plots without P fertilization (R^2 = 0.92, *P* = 0.027, **Figure 3.4**).



884

885 **Figure 3.4**. Relationship between plant functional traits (± standard error, x-axis) and
886 aboveground net primary production (ANPP) (± standard error, y-axis) by species (symbols)
887 and treatments (colours). Dashed lines (RTD and SRL) indicate a significant relationship
888 between the trait and ANPP for *Cynodon dactylon* (symbol = circle).

889

3.3.4 Stomatal Conductance

Repeated measures of g_s are presented in **Figure 3.5**, showing species differences in the rate of recovery of stomatal function following watering events. In *C. dactylon*, interactive effects of P addition and reduced rainfall were apparent during the first three samplings (**Figure 3.5a**), with phosphorus addition alleviating the negative effects of drought g_s for this species. We observed a similar pattern in g_s for *E. curvula*. For this species, overall g_s was slower to rise but was maintained after soil moisture had begun to decline following the largest rainfall inputs on days 19 and 20 (**Figure 3.5b**). Stomatal conductance (g_s) in *M. stipoides* was enhanced by P addition; the overall interaction between P and rainfall treatments was not significant, although on one (day 31) of the seven days when this was measured there was a significant treatment interaction for this species (**Figure 3.5c**). For *S. parviflora*, the only interactive treatment effects were on the first sampling day when g_s was minimal (**Figure 3.5d**). While P addition resulted in significantly greater g_s, there was no evidence that it alleviated the negative effects of reduced soil moisture as was seen for *C. dactylon*.

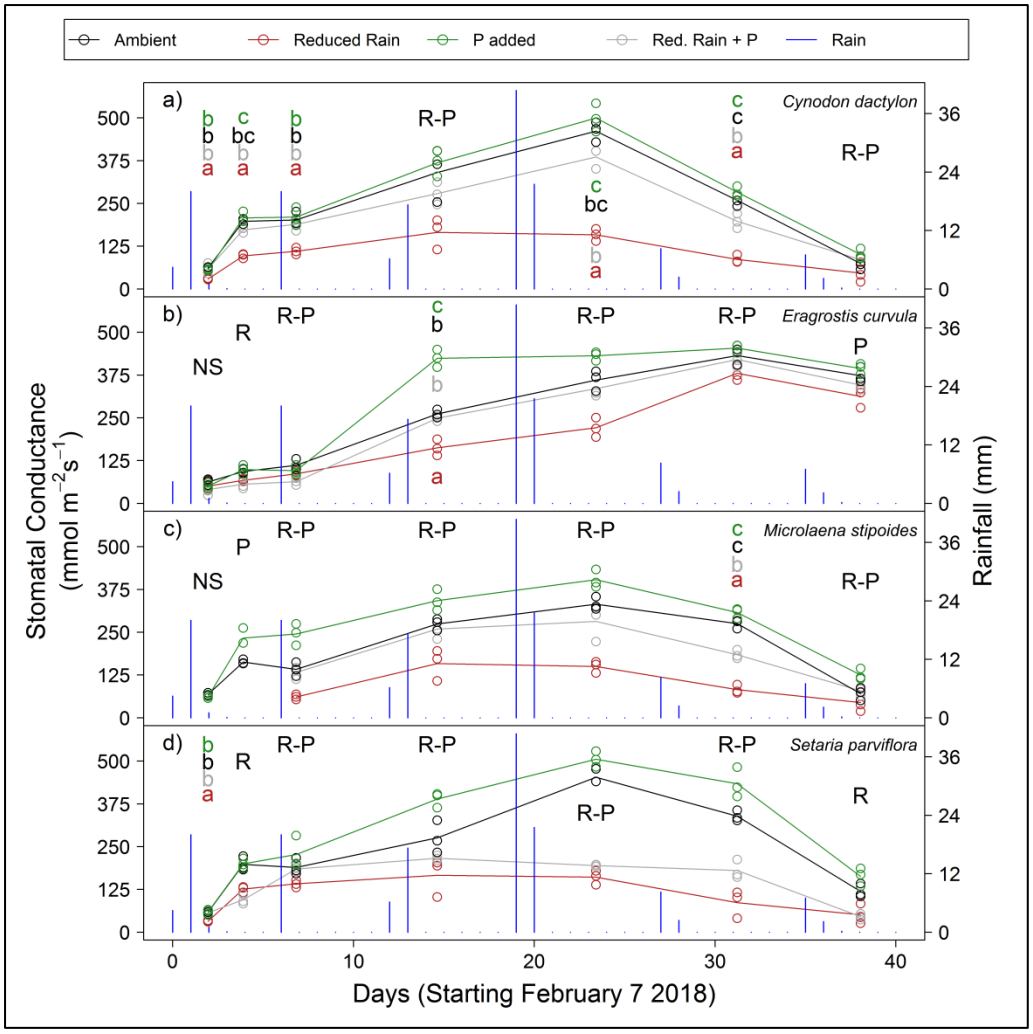
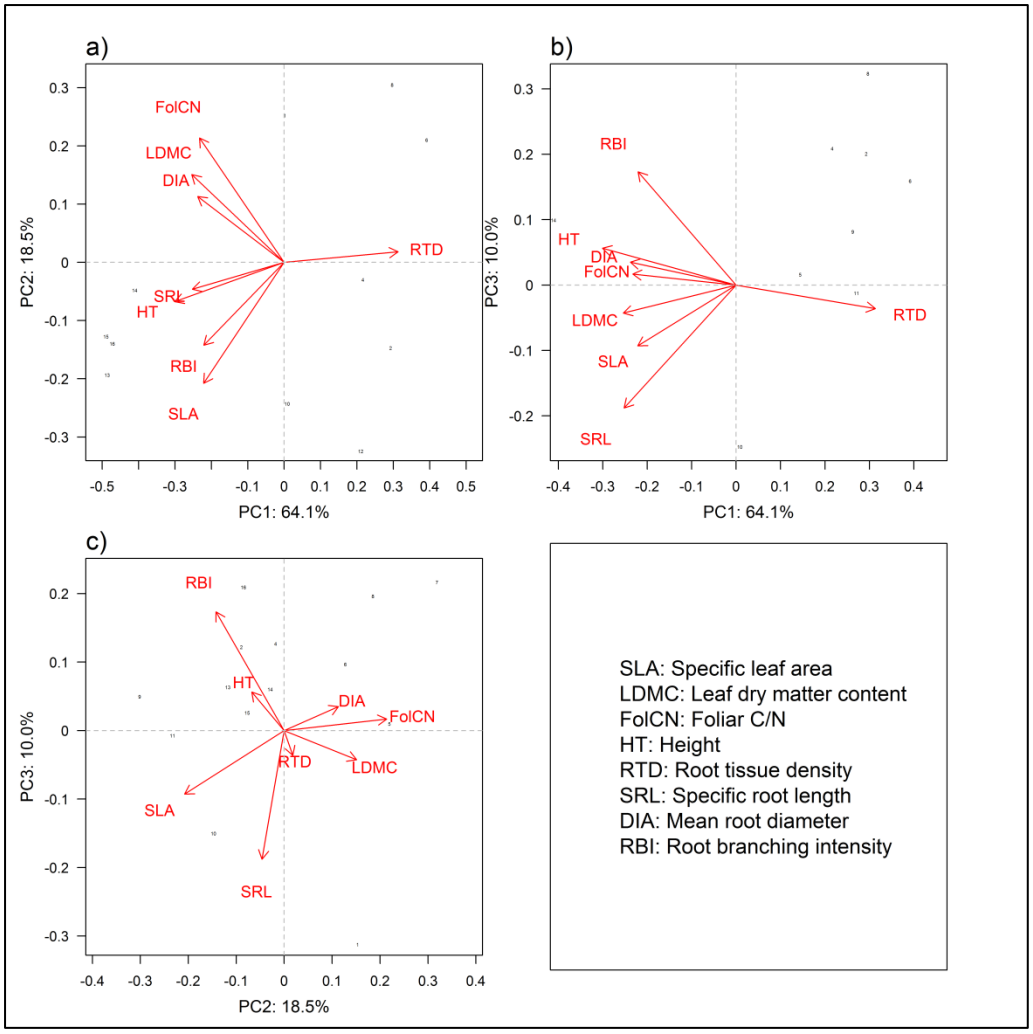


Figure 3.5. Stomatal conductance for (a) *Cynodon dactylon*, (b) *Eragrostis curvula*, (c) *Microlaena stipoides* and (d) *Setaria parviflora* following late summer precipitation events. Significant effects of rainfall treatment are denoted by "R" within a given sampling date, while significant effects of phosphorus treatment are denoted by "P". "R-P" indicates each treatment had a significant effect (i.e. non-interactive). Coloured letters indicate a significant interaction between rainfall and phosphorus treatments, with letters corresponding to treatments (as indicated by colour), following Tukey single-step post hoc analysis. "NS" denotes no significance.

3.3.4 Principle Components Analysis

The first three PCA axes explained 64.1%, 18.5% and 11.2% of total variation of species and treatment-specific traits. Three unique groups separated along the first two axes (**Figure 3.6a**). Root tissue density (group 1) was isolated and strongly associated with the first PCA axis. Foliar C:N, LDMC and DIA (group 2) were negatively associated with RTD (**Figure 3.6a**). The remaining traits (SRL, HT, RBI and SLA; group 3) were also negatively associated with RTD but appeared to separate from group 2 along the second axis (Figure 3. 3. 6a). When using PC axes 1 and 3, the independence of RTD was still apparent, although the remaining traits re-aligned into one group (**Figure 3.6b**). An ordination of PC axes 2 and 3 demonstrated greater independence of traits, although coordination of a few traits was still observed (**Figure 3.6c**). Root diameter (DIA), LDMC and FOLCN appeared related along the first axis (group 1). Plant HT and RBI were negatively associated with group 1 along the second axis but appeared coordinated along the third. Specific root length (SRL) appeared independent of these groups, with only minimal association with SLA or RTD (**Figure 3.6c**).



932

933 **Figure 3.6**. Principle components (PC) analyses highlight independent axes of plant
934 functional traits in four grassland plant species treated with four years of P fertilization and
935 three years of rainfall reduction. Axes one and two (panel a) explain 64.1% and 18.5% of
936 variation in the data, respectively, while axis three (panel c) explained 10.0% of the data
937 variance. Axes one and three are presented in panel b.

938

3.4 DISCUSSION

In this study, we addressed five questions regarding how drought and soil P addition affect ecosystem functioning (ANPP) and associated traits of the dominant species in a mesic grassland. Reduced rainfall reduced ANPP by 29% while P addition increased ANPP by 62%. We found no interactions between P addition and drought treatments in terms of total plant ANPP, indicating P addition did not alleviate the negative effects of drought on grassland productivity which answers our first question. We did, however, observe enhanced recovery of g_s with P addition, following re-wetting, in three of the four dominant species sampled. These changes were concurrent with changes in RTD of these three species; however, only for *C. dactylon* were higher values of RTD in P addition plots associated with greater ANPP. Therefore, we partially answered our first hypothesis as there is evidence to suggest that P addition does provide some physiological benefit to drought-stressed grasses, but only in specific species, and which did not translate to greater ANPP. The topic warrants further investigation as studies elsewhere have found the hypothesis to be true for other plant species and ecosystems (Nelsen and Safir 1982; Rodriguez et al. 1996; Garg et al. 2004; Jun et al. 2017). Since soil nutrients and rainfall are the primary factors controlling grassland maintenance and productivity (Suttie et al. 2005; Guo et al. 2012; Fay et al. 2015), it is feasible that systems where co-limitation exists may benefit the most from P addition under future rainfall scenarios, via enhanced root productivity (Hajabbasi and Schumacher 1994) with shifts towards more (e.g. *M. stipoides*) or less (e.g. *C. dactylon*) acquisitive strategies.

We found that ANPP of multiple plant species in a single community did not respond similarly to reduced water availability and P addition, answering our second question. In fact, at the species level, only *S. parviflora* decreased ANPP under reduced rainfall and only *C. dactylon* increased ANPP with P addition. So with respect to our second question, we can conclude that *S. parviflora* is the species most sensitive to extreme drought in our system.

965 Potentially, because shelters had been in place since mid-2015 with short-periods of water
966 deficit over that period it is possible that species other than *S.* parviflora had acclimated to
967 low water conditions. This could explain why *S. parviflora* was the only species to have a
968 negative response to reduced water. As the only species which was unaffected in its trait
969 values under any of the treatments, it may be that its inability to acclimate to low soil water
970 availability or capitalize on increased nutrient availability accounts for its loss of productivity
971 under dry conditions. In contrast, the positive effect of P addition on ANPP of *C. dactylon*,
972 and on four of its eight measured traits suggests that species that readily acclimate to changes
973 in resource-availability may do best under future changes in rainfall regimes and associated
974 soil water stress. Fort et al. (2015) found acquisitive strategies in legumes were associated
975 with reduced negative effects of water and P limitation; however, our observations in *C.*
976 *dactylon* contradict their finding. Others have found P deficiency to decrease root hydraulic
977 conductance per root surface area in small trees (Trubat et al. 2006), which could have
978 negative implications for drought tolerance. This suggests there may be distinct differences in
979 responses of differing plant functional types to drought and fertilisation (Baldocchi et al.
980 2004; Volaire 2008; Onipchenko et al. 2012).

981 We found strong interspecific differences amongst our dominant grasses with respect
982 to changes in trait responses to reduced rainfall and P addition, answering our third question.
983 While many traits were unaffected by drought or P addition, we observed contrasting
984 directional responses of traits across species. For example, SRL increased with P addition in
985 *M. stipoides*, but decreased in *C. dactylon*. While we attribute these changes to the direct
986 effects of P addition, we did not investigate the indirect effects (Soliveres et al. 2015). For
987 example, with limited niche space for root exploitation, a direct effect of P addition on SRL
988 of *C. dactylon* could result in a shift in root traits of *M. stipoides*, via competition. In addition,
989 unique P requirements between *M. stipoides* and *C. dactylon* could result in opposite

990 responses to fertilisation (Lambers et al. 2006). This highlights the complexity of trait-
991 environmental relationships in complex plant communities and warrants further study, ideally
992 through the use of coordinated experiments (Fraser et al. 2013; Ploughe et al. 2018).

993 Of the four species surveyed, only one (*C. dactylon*) had changes in trait values (for
994 RTD and SRL) that were associated with changes in ANPP, partially answering our fourth
995 question. Interestingly, greater ANPP was associated with more conservative values of both
996 RTD (greater density of root tissues) and SRL (less root length per mass). It is possible that
997 greater resource availability led to a less acquisitive resource-use strategy leading to reduced
998 tissue water loss in roots (Louise Comas et al. 2013) and allowing for greater ANPP in the
999 field; however, this was not tested directly (Manschadi et al. 2006). Alternatively, changes in
1000 the ratio of roots to shoots can affect productivity under drought (Akram et al. 2007).
1001 Because of the heterogeneous nature of mesic grasslands, determining belowground biomass
1002 by species is difficult, although this would clearly benefit from further investigation. We can
1003 assume that the response of *C. dactylon*, which is the most dominant species (>50% of all
1004 ANPP), and its associated traits, will likely dictate community-level responses to future
1005 change (i.e. the biomass-ratio hypothesis) (J. P. Grime 1998). Other important traits relating
1006 to water and nutrient uptake, such as rooting depth and root mass fraction, could explain why
1007 some species performed better than others under reduced rainfall. However, such measures
1008 are difficult to undertake in mixed field communities, are highly destructive, and not suited to
1009 long-term experiments.

1010 The significant treatment effects on RTD and the relationship between RTD and
1011 ANPP for one of the four species identifies this as a key trait determining ANPP in the plant
1012 community. The importance of this trait was confirmed in the multivariate analysis as RTD
1013 was independent of all other traits along the first principal component axis, which explained
1014 64.1 % of the data variance. The importance of RTD as an trait indicative of plant resource-

use strategy has been well-documented (P. Ryser 1996; Birouste et al. 2014; Kramer-Walter et al. 2016). Overall, our results provide conflicting evidence in relation to the plant economic spectrum in our mesic grassland flora. Other than identifying RTD as a key trait in our system, we observed limited coordination across the first three PC axes, answering our fifth question regarding the extent of coordination in the community. There were, however, two exceptions to this. First, SLA and SRL were consistently co-located in plots of the first three PC axes. SRL appears not to be useful in trees due to its varying nature of correlation amongst other traits (Kramer-Walter et al. 2016) and SLA is highly variable (Wilson et al. 1999), which may indicate these traits are coordinated but related to resource foraging ability rather than plant investment trade-offs. A second trait grouping, comprising FolCN, LDMC and DIA, was also apparent. This group of traits therefore likely encapsulates the plant economic spectrum in our system. However, since these traits were unrelated to ANPP, we are unable to confidently apply the concept of conservative and acquisitive plant strategies to understand the ecology and explain the performance of species in our study. Others have observed similar species-specific changes in these traits under fertilisation (Leuschner et al. 2013); however, the abundance of traits (associated with physiological strategies) has been shown to be correlated to plant growth rates (Poorter and Remkes 1990). In our study, RTD was the key trait underlying ANPP but only for a single species.

3.5 CONCLUSION

Five hypotheses were tested regarding responses of a mesic grassland to severe drought, the role of P fertilisation in alleviating drought effects and the mechanisms (traits) underpinning plant responses. Although drought resulted in a reduction in ANPP for one of the four species examined, P addition did not alleviate this effect, despite significant effects of this nutrient on traits. Interspecific differences in morphological, chemical and physiological traits of co-

occurring perennial grassland species were important to explain treatment-driven changes in ANPP. We found RTD to be an important trait underlying responses of ANPP in *C. dactylon* species. However, P addition had contrasting effects on RTD across the dominant species sampled. Overall, species that experienced significant changes in traits in response to P addition (i.e. high trait plasticity) were least affected by drought, while species whose traits were unchanged by P addition were most sensitive to water stress. In the single most abundant species, changes in root traits indicative of a shift towards more conservative resource strategies were associated with greater productivity. In conclusion, soil P fertilisation has the potential to increase grassland drought tolerance through changes in key traits related to productivity under reduced water availability, although we did not observe a direct effect of drought alleviation by P addition in this study.

Chapter 4: Allometric relationships to estimate aboveground biomass are improved by increasing specificity of plant functional groups

In review with *Journal of Arid Environments*

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AUTHOR CONTRIBUTIONS: JC conceived the ideas, acquired data, analysed data and wrote the manuscript. UN designed the experiment and all authors contributed critically to manuscript development.

4.0 ABSTRACT

Plant aboveground biomass (AGB) is a useful metric to assess ecosystem health and functioning, and its sensitivity to changing environmental conditions provide insight into potential global change impacts. Allometric estimates of AGB using a non-destructive proxy such as plant cover or height can be useful for repeated quantification over time, but can introduce uncertainty to estimates. We estimated the relationship between both plant *cover* and a *cover·height index* and AGB for fifteen plant species from six sites across semi-arid rangelands in eastern Australia to select the best approach. Estimates of AGB were based on four categorizations to test if generic estimates were more robust than grouping species based on life-history and morphology (i.e. categories based on plant functional types). We found that the *cover·height index* was superior to the use of *cover* alone in estimating AGB. Although increasing specificity of plant groupings increased the robustness of *cover-AGB* and *index-AGB* relationships, we found that estimates did not perform equally well across sites. AGB was consistently overestimated across the six sites when using *cover* only (63.9 ± 48.5 percent error between observed and predicted biomass amounts). AGB was consistently over-estimated (63.2 ± 39.0 percent error) at three of the six sites and under-estimated (-58.1

± 9.1 percent error between observed and predicted biomass amounts) at the remaining three sites when using the *cover·height index*. Whilst broad allometric relationships validated on the basis of plant functional group data can provide a coarse approximation of AGB at the landscape scale, development of new species-specific allometric estimates incorporating additional time-intensive measurement proxies may be necessary.

4.1 INTRODUCTION

Robust measurements of aboveground net primary productivity (ANPP, g m^{-2} $year^{-1}$) are critical for monitoring ecosystem sensitivity to climatic variability (Knapp and Smith 2001; Scurlock et al. 2002), and calculating landscape level carbon stocks or global carbon dynamics (Le Quéré et al. 2015). In many plant communities, the use of destructive sampling to obtain ANPP data can result in permanent loss of harvested individuals with substantial consequences for the ecology of the system (Guevara et al. 2002; Flombaum and Sala 2007; Montès 2009). Destructive sampling is also time-consuming, costly, and especially in remote locations, there are challenges to transporting and processing large amounts of material. The strategy for estimating AGB can vary depending on the plant community and the factors regulating productivity (Sala et al. 1988; Biondini et al. 1991). For example, grasslands typically undergo destructive harvests either at peak biomass or several times throughout the year (seasonal peak biomass) (Singh et al. 1975; Sala et al. 2000). Repeating these measurements over time may alter species composition by selecting plants that are not affected by clipping (e.g. perennial grasses) rather than those that are impacted negatively (e.g. shrubs). In Australia, where rainfall is expected to become highly variable, estimates of changes in AGB (ANPP = $AGB_{T2} – AGB_{T1}$) will become increasingly important to quantify global carbon dynamics (New et al. 2001; Haverd et al. 2016). In addition, Australia's economic output is tied to the country's vast rangelands and grazing management (Bird et al.

1992; McAlpine et al. 2009). Developing sound methodology to rapidly quantify AGB in shrub and herbaceous-dominated plant communities, and how it changes in response to altered rainfall patterns and climate more generally, is crucial to protecting such enterprises.

The development of allometric estimates (using equations with both an intercept and slope) of AGB is underpinned by two key decisions (Paul et al. 2016). The first is to determine which predictor variables (or plant attributes) to correlate with standing AGB. In forests, the established paradigm of using stem diameter, stem height, and wood density has proven accurate in predicting AGB (Chave et al. 2014; Picard et al. 2012; Picard et al. 2015). In contrast, cover-based and volumetric methods are widely used for predicting AGB in grass- and shrub-dominated ecosystems (Huenneke et al. 2001; Flombaum and Sala 2007; Nafus et al. 2009). The cover-based method uses linear regression to determine the relationship between vegetation cover (predictor variable) and standing AGB (unknown dependent variable), where the intercept is typically set to zero (i.e. no cover equals no biomass). However, obtaining a sufficient number of data points along the vegetation cover axis may require removal of portions of a plant to obtain the required 'spread' (i.e. to include low values), which may increase uncertainty in estimates (Montès 2009). The volumetric method uses linear regression to estimate AGB (y-axis) from volume data derived by multiplying cover by height (x-axis). The calculated volume is not of the plant itself, but rather a rectangular box in which the plant is hypothetically encased. The slope of the linear relationship between AGB (dependent variable) and either volume (predictor variable) or cover (predictor variable) can then be used to non-destructively estimate AGB at a given site using *cover* only or *cover·height index*.

Several studies have used cover, sometimes in combination with height, to estimate the impact of year-of-sampling or location on the measurement-AGB relationship. For example, in a semi-arid herbaceous system in Central Hungary (mean annual precipitation of

~500 mm), the relationships between visually estimated plant cover and AGB varied between years and was increasingly positive (greater AGB per unit cover) with greater amounts of rainfall preceding harvests (Ónodi et al. 2017); however, the authors did not use height in their estimates. Others have found the addition of height did not significantly affect AGB estimates in grass-based communities (Andariese and Covington 1986; Assaeed 1997; Guevara et al. 2002; Nafus et al. 2009). Since above-average rainfall can result in greater AGB production and associated increases in light-limitation (Knapp 1984), the inclusion of height may be beneficial, and provide a mechanistic link, for developing robust allometric estimates to be used in longitudinal studies within herbaceous plant communities, even if the addition does not aid in AGB estimates at a single time point. In addition, plant height may be indicative of competitive vigor (Cornelissen et al. 2003), which is likely to vary given environmental heterogeneity (Menaut and Walker 2001). The natural variation in height found in a plant community may provide a sufficient 'spread' along the x-axis for robust regression analysis of plant attribute-AGB relationships.

The second key decision to make is what level of specificity (e.g. species, genus, functional group) is needed to develop robust allometric estimates (Paul et al. 2016). In other words, are plant attribute-AGB relationships for every species required or can plant species of similar form and/or habitat be grouped to determine the slope between plant attributes and AGB? Paul et al. (2016) tested this concept in Australia utilizing 274 woody plant species (trees and shrubs) across 826 sites, and concluded generalized allometric equations did not present substantial bias or inaccuracy in predicting AGB. This result suggests that the way species are grouped and their home eco-region (habitat based on temperature and precipitation) is possibly arbitrary for estimating AGB. Similarly, Nafus et al. (2009) found that multispecies allometric estimates (using basal diameter and height) of eight grass species were robust in the semi-desert rangelands of Arizona. However, unlike Paul et al. (2016),

Nafus et al. (2009) cautioned against using the biomass-size relationships at other sites because inter-site variation in allometric relationships has been observed across broad plant functional groups (e.g. deciduous trees, shrubs, grasses) (Andariese 1986; Buech and Rugg 1989; Ares and Fownes 2000). In arid and semi-arid herbaceous communities (e.g. grasslands), pulse-rainfall patterns may result in periods where communities fluctuate between water-limited (light abundant) and light-limited (water abundant), thereby leading to changes in vegetation height (i.e. greater plant height with increasing light limitation) (Knapp 1984). If so, the addition of height to allometric estimates may permit cross-site use of allometric estimates of AGB.

A common theme amongst allometric studies has been the potential trade-off between species-specific and multispecies estimates (**Figure 4.1**) and determination of species groupings (Nafus et al. 2009; Paul et al. 2016; Vieilledent et al. 2012; Fayolle et al. 2013; Conti et al. 2013). Coarse characteristics of plant groups (woody *vs.* herbaceous, shrub *vs.* grass) may be ideal to address this issue as assignment and classification of plants in the field would be rapid. Communities can be described as being composed of forbs, grasses, shrubs and trees. A key issue for ecologists in the field is therefore how to optimize the use of species groupings in the determination of biomass allometry relationships. Ishihara et al. (2015) investigated this topic and found generic equations (i.e. equations across groupings) were sufficient in predicting AGB in a Japanese forest, even when the plant attribute-AGB relationships were developed off-site. They found broad plant groups (deciduous, evergreen, angiosperm, gymnosperm) were useful. These criteria, however, fail to capture the physical structure of grass- and forb-dominated communities, indicating that unique levels of specificity and plant groupings are required for allometry in herbaceous systems, depending on the habitat type and resolution of interest.

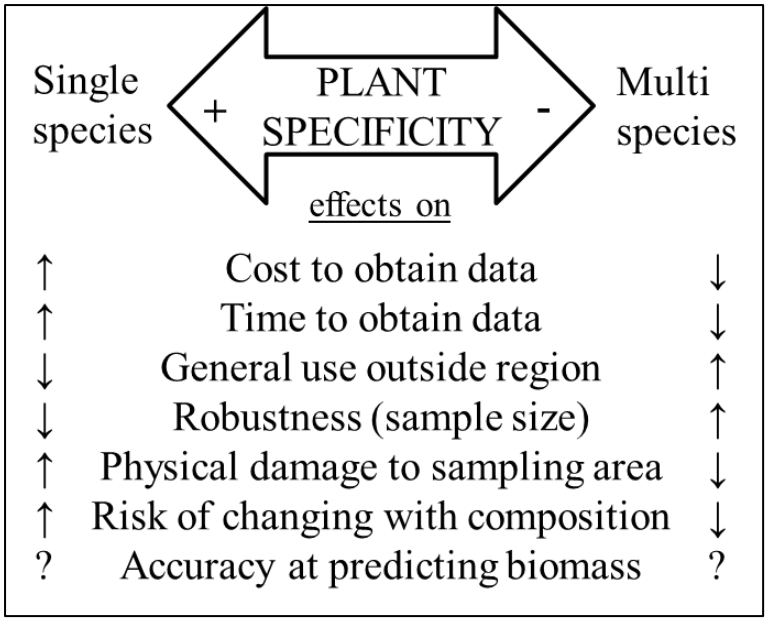

Figure 4.1. A summary of how specificity of plant groupings represents trade-offs for generating allometric estimates of biomass. Single species (high specificity) and multispecies (low specificity) allometric estimates logically have trade-offs. Arrows indicate how each performance criteria might change with plant specificity. Developing allometric estimates for individual species can increase cost and time to obtain data. In addition, the use of these estimates outside the collected region (i.e. off-site usage) may be limited and the overall number of samples obtained (robustness) will be restricted. Individual species estimates, therefore, will require a greater number of samples, resulting in greater damage to the sampling area. In addition, if a species no longer exists at that site over time (say through the implementation of a treatment) there is a chance that the allometric equation will no longer be useful. Less is known about how species-specific and multispecies allometric estimates compare at accurately predicting actual biomass.

The aim of this project was to develop a simple and rapid methodology to estimate AGB across eastern Australian rangelands using broad plant functional groups (herein categories). More specifically, we wanted to 1) determine if the inclusion of height (using *cover·height index*) improved AGB estimates relative to using *cover* alone; 2) determine the optimum level of specificity for plant groupings that maximizes the accuracy of allometric estimates of AGB; and 3) investigate if the accuracy (percent error) in estimated ABG biomass was similar across each of the six sites used for plant collection. We hypothesized that the addition of height would improve estimates of AGB across all levels of specificity since 'height' and 'plant strategy' are related and 'plant strategy' and 'functional groups' are also likely to be related. In addition, we hypothesized that the accuracy of AGB estimates would be highest when using more specific plant categories. Lastly, we hypothesized that the accuracy of the allometric estimates (tested against destructively harvested calibration plots) would vary across sites because of differences in species diversity and abundance at each site.

4.2 Materials and Methods

4.2.1 Sites and Species Selection

Six field sites were selected as part of the ecological research project "Dryland Rainfall Legacies" (DRL) investigating the effect of historic rainfall patterns on ecosystem sensitivity to rainfall variability, established in September 2016. The six sites vary in 10-year annual rainfall amounts and in their inter-annual variability in rainfall over that period (**Table 4.1**). Nine permanent vegetation plots (1.5 m^2 each) were surveyed for plant cover using the Daubenmire method (Daubenmire 1959) with a gridded quadrat (1.5 m^2) at each site (54 plots total). Plant species for allometric calculations were selected based on the most abundant species at that site and the availability of individual plants outside the permanent plots.

Table 0.1. Sites, locations and rainfall characteristics. Sites are paired by mean annual precipitation but vary in inter-annual variation (CV = mean/standard error).

Site name (and state)	Coordinates	Mean Annual Precipitation (mm)	Coefficient of Variation (CV)	Standing Biomass ($g \cdot m^2$)[$]
Broken Hill, NSW	31°58'33.2"S, 141°33'19.3"E	251	0.28	215.0
Milparinka, NSW	29°36'21.2"S, 141°43'01.9"E	265	0.52	268.5
Cobar, NSW	31°47'45.0"S, 145°35'46.7"E	367	0.27	397.1
Quilpie, QLD	26°34'38.1"S, 144°37'08.5"E	356	0.57	147.1
Nyngan, NSW	31°38'42.7"S, 146°38'30.8"E	466	0.31	523.1
Charleville, QLD	26°21'53.1"S, 146°09'02.6"E	442	0.53	217.4

Precipitation data from the Bureau of Meteorology (http://bom.gov.au), averaged from 2002-2012. [$]Data obtained from 'calibration plots' located outside the permanent plot area.

4.2.2 Data Collection

Based on vegetation surveys and availability of plant material, fifteen species were selected for this study and biomass data were collected in September 2016 (experiment initiation, beginning of spring) and March 2017 (end of summer) (**Table 4.2**). Data were grouped into three categories, based on observations in the field and species information in the literature (Cunningham et al. 2011). Category A is the broadest, distinguishing two groups: *herbaceous* (12 species) and *woody* (3 species). Category B uses phylogeny and primary growth form to distinguish three groups: *forb*s (8 species), *grasses* (4 species) and *woody* s*hrubs* (3 species). Category C is the most specific with six groups: *leafy woody shrubs* (1 species), *sclerophyllous woody shrubs* (2 species), *forbs* (3 species), *sub-shrubs* (4 species), *long-lived grasses* (2-3 species, see below) and *short-lived grasses* (2 species). *Sub-shrubs*, or bushes, were considered to be plants that may have woody components (particularly at the base), but where the majority of growth (stems and branches) is herbaceous (B. D. Jackson 1905). *Long lived* and *short-lived* grasses were determined based on the presence of a 'crown' when foliar die-back occurred in drier periods. All four grass species sampled are perennial; the persistence of a crown indicates that it is the same individual 're-growing' when rainfall occurs following a dry-period (Cunningham et al 2011). Long-lived grasses, *Astrebla lappaceae*, *A. pectinata* and *Eragrostis setifolia*, were found to have persistent crowns during the second sampling in March 2017, while the remaining two species did not. Both *Astrebla* species were combined for analyses because they have nearly identical growth forms (Cunningham et al 2011) and are found at the same site (Milparinka).

Table 0.2. Details for collected species including sample size (n) and categories (A = broadest, B = intermediate; C = most specific). 'Range of cover values' indicates the size of individuals that were used to develop allometric estimates.

Species	Family	n	Site	Collected	Category A	Category B	Category C	Range of cover values [min-max] (cm^2)
Astrebla lappaceae (Lindl.) Domin; *A. pectinata* (Lindl.) F.Muell. *ex* Benth.	Poaceae	40	Milparinka, NSW	March 2017	Herbaceous	Grass	Long life grass	24-3240
Eragrostis setifolia Nees.	Poaceae	30	Quilpie, QLD	March 2017	Herbaceous	Grass	Long life grass	25-696
Austrostipa scabra (Lindley) S.W.Jacobs & J.Everett	Poaceae	10	Nyngan, NSW	March 2017	Herbaceous	Grass	Short life grass	4-400
Thyridolepis mitchelliana (Nees) S. T. Blake	Poaceae	30	Nyngan, NSW	March 2017	Herbaceous	Grass	Short life grass	1-64
Calotis lappulacea Benth.	Asteraceae	30	Nyngan, NSW	March 2017	Herbaceous	Forb	Forb	6-810
Ptilotus sessilifolius (Lindl.) Benl	Amaranthaceae	30	Cobar, NSW	March 2017	Herbaceous	Forb	Forb	8-713
Rhodanthe floribunda (A.Cunn. *ex* DC) Paul G. Wilson	Asteraceae	25	Milparinka, NSW	March 2017	Herbaceous	Forb	Forb	20-840
Abutilon halophilim F.Muell.	Malvaceae	15	Milparinka, NSW	March 2017	Herbaceous	Forb	Sub-shrub	418-3480
Atriplex vesicaria Heward(es; fr) ex Benth.	Amaranthaceae	20	Broken Hill, NSW	September 2017	Herbaceous	Forb	Sub-shrub	144-1215
Sclerolaena eriacantha (F.Muell.) Ulbr.	Chenopodiaceae	25	Quilpie, QLD	March 2017	Herbaceous	Forb	Sub-shrub	25-486
Sclerolaena ventricosa (J.M.Black) A.J.Scott	Chenopodiaceae	15	Broken Hill, NSW	September 2017	Herbaceous	Forb	Sub-shrub	135-897
Sida petrophila F.Muell.	Malvaceae	20	Broken Hill, NSW	March 2017	Herbaceous	Forb	Sub-shrub	1-676
Eremophila gilesii F.Muell.	Scrophulariaceae	30	Charleville, QLD	March 2017	Woody	Shrub	Leafy	462-9800
Maireana pyramidata (Benth.) Paul G. Wilson	Chenopodiaceae	15	Broken Hill, NSW	March 2017	Woody	Shrub	Sclerophyllous	228-7875
Maireana sedifolia (F.Muell) Paul G. Wilson	Chenopodiaceae	15	Broken Hill, NSW	September 2017	Woody	Shrub	Sclerophyllous	104-2332

Only healthy, undisturbed and relatively isolated individuals (to minimize potential neighbor effects; see Montès 2009) were sampled for estimation of AGB. Plant basal area was calculated from cover (Daubenmire 1959) by multiplying the cover percentage by the area of the quadrat at ground level. Individual stem/tiller height, from ground level to tallest leaf collar (i.e. vegetative height) was recorded to the nearest 5 cm (Guevara et al. 2002; Nafus et al. 2009). The *cover* (m^2) and *cover·height index* (m^3) of each plant were used to estimate AGB. The whole aboveground portion of the plant was harvested using hand-pruners and stored in paper bags. Samples were subsequently dried at 60 °C for 72 hours and weighed. At each site, one 'validation plot' (1.5 m^2) (see **Table 4.1**) was used for subsequent 'accuracy' testing of the allometric estimates based on data collected from the individual plants. Each validation plot was assessed for cover (Daubenmire 1959), measured for vegetative height using the tallest individual of each species, then harvested in its entirety to ground level. The $AGB_{Validation}$ was sorted out by species, dried and weighed separately. *Cover* and *cover·height index* data are presented in the units of m^2 and m^3, respectively.

4.2.3 Data Analysis

Data for creating *cover*-AGB and *cover·height index*-AGB estimates were analyzed with linear regression in R (R Studio V1.0.143, R V3.2.5, R Core Team 2013) using the '*lm´* function. The scatterplots for linear assumptions are presented in **Figure S4.1** and **S4.2** for *cover* and **Figure S4.3** and **S4.4** for *cover·height index*. The intercept of the linear model (**Equation 4.1**) was set to zero, because zero plant cover would equal zero AGB, and each species included a '0 *cover*/*cover·height index* and 0 AGB' data point to reflect this (Flombaum and Sala 2007). Data were not transformed.

Equation 4.1: $y = mx + b$

For **Equation 4.1:** *y* is equal to AGB, *m* is equal to *cover* or *cover·height index*, *m* is equal to the slope (relationship between *y* and *cover* or *cover·height index*), and *b* is equal to the y-axis intercept (which was set to zero). The slope (*m*), therefore, is equal to the change in AGB given a change in either *cover* or *cover·height index*. Alpha was set at 0.05. This equation was calculated for: 1) all species; 2) category A (2 groups); 3) category B (3 groups); and 4) category C (6 groups). Model selection was based on comparison of adjusted R^2 values (**Equation 4.2**), Akaike's information criterion (AIC) (**Equation 4.3**), and root mean square error (RMSE) (**Equation 4.4**).

$$\textbf{Equation 4.2a: } R^2 = \frac{SS_{residuals}}{SS_{total}}$$

$$\textbf{Equation 4.2b: } Adjusted\ R^2 = 1 - \frac{(1 - R^2)\,(n-1)}{n-p-1}$$

$$\textbf{Equation 4.3: } AIC_i = -2 \cdot log(L_i + 2V_i)$$

$$\textbf{Equation 4.4: } RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Est_i - Obs_i)^2}$$

For **Equations 2** through **4**, *n* represents the sample size. For **Equations 4.2a** and **4.2b**: *p* is the number of predictor variables, $SS_{residuals}$ is the sum of errors, and SS_{total} is the total variance. For **Equation 4.3**: L_i is the maximum likelihood for the candidate model *i*, and V_i is the number of parameters (i.e. factors) estimated from the data for the model *i* (Akaike 1998). Models with lower AIC were considered better estimates. The rules we applied regarding the

use of AIC for model selection were that: 1) models with a difference of less than two should both be considered for selection; and 2) models with a difference of greater than 10 should be omitted (in preference of the lowest AIC) (Burnham and Anderson 2003). Models with a difference in AIC between 2 and 8 are not significantly different and should be chosen based on the intended usage. For **Equation 4.4**: Est_i and Obs_i are the estimated and observed biomass for sample *i*, respectively, and *n* is the total number of samples.

The percent change ([final-initial] / [initial] * 100) was calculated for the adjusted R^2 and RMSE, where *cover* based values were considered the initial and *cover·height index* values were final. In other words, a positive percent change would indicate that the addition of height increased the adjusted R^2 or RMSE. It is important to note that increased adjusted R^2 means greater amounts of variation were explained by the model and increased RMSE means less variation was explained by the model. Improvement in AIC was calculated as the *cover* AIC minus the *cover·height index* AIC, meaning a positive value indicates that adding height improved AGB estimates. In all cases there was only one predictor value (*cover* or the volume from the *cover·height index*) which would not introduce bias to adjusted R^2 or AIC values.

Slopes (change in AGB per unit *cover* or *cover·height index*) were used to estimate AGB using cover and height data obtained from the validation plots. This was done using the 'all species' and category-based equations for both *cover* and *cover·height index* values. The accuracy of predicted (model-based) AGB data was determined by comparison with data from all validation plots, and then for each site individually. The percent error was calculated by dividing the difference between $AGB_{Observed}$ and $AGB_{Predicted}$ by the $AGB_{Observed}$ value. This was calculated using validation plots from each site individual, as well as the pooled data from six plots (one plot per site).

4.2.4 Cover- and Cover·height Index-AGB Allometric Model Selection

The question of how to select the most appropriate equation or model fit remains a key issue when conducting allometric estimates of biomass across multiple taxa and lifeforms (Chave et al. 2004; Zhu et al. 2009; Chen et al. 2009; Molto et al. 2013). Several different approaches have been reported in the recent literature. For example, Chave et al. (2014) used bioclimatic predictors of AGB that led to the lowest residual standard error (RSE) while previously reporting RSE and AIC in estimates of AGB in a tropical forest (Chave et al. 2005). For high elevation grassland plant species, Oliveras et al. (2014) reported adjusted R^2, RMSE and AIC. In a broader allometric assessment of AGB of trees, shrubs and herbaceous plants, Ensslin et al. (2015) selected proxy-AGB fits based on AIC, but still reported adjusted R^2.

For the purpose of this study, we chose to report AIC, adjusted R^2 and RMSE. The use of AIC to select allometric models is well established (Burnham and Anderson 2003). Adjusted R^2 is highly comparable across prediction parameters and is commonly used for allometric estimates of AGB. Reporting RMSE is common and represents a biologically relevant approach to compare allometric equations. For example, if the *cover*-AGB relationship estimate is 500 $g \cdot m^2$, with a RMSE term of 50 $g \cdot m^2$, we could say that 50% plant *cover* would be 250 ± 50 $g \cdot m^2$ of AGB. The ratio of RMSE to the slope is useful because it allows comparison across groups with unique slopes and error terms. For example, a RMSE of 50 with a slope of 100 (50/100 = 50% of the estimated value) would be superior to a RMSE of 10 if the slope is 16 (10/16 = 62.5% of the estimated value). All three metrics were used to determine whether height (i.e. *cover·height index*) improved allometric estimates of AGB. Adjusted R^2 and RMSE were considered as a % increase (better) or % decrease (worse) without a threshold to determine whether the % change was 'significant.'

4.3 RESULTS

4.3.1 Overall Model Fit Summary

All regression results were highly significant ($P < 0.001$) with adjusted R^2 values ranging between 0.38 and 0.98 with the deviation of observed and predicted values depending on the caterogy and species (**Figure S4.5** and **Figure S4.6**). The slope for all species combined using cover was 830 $g \cdot m^2$ (**Table 4.3**), while *cover·height index* was 1556.7 $g \cdot m^3$ (**Table 4.4**). Using category A (*herbaceous* vs. *woody*) there was a small difference in the *cover·height index* estimate between woody and herbaceous species (1562.0 and 1472.3 $g \cdot m^3$, respectively), while the estimates diverged using *cover* only (863.5 and 590 $g \cdot m^2$, respectively) (Table S.

Table 0.3. Slopes, adjusted R^2, root square mean errors (RMSE), the RMSE/slope ratio and Akaike's information criteria (AIC) for plant categories and species tested for *cover*-aboveground biomass relationships. All relationships are highly significant ($P < 0.001$).

Category/Binomial		Slope ($g \cdot m^2$)	Adjusted R^2	RMSE ($g \cdot m^2$)	RMSE/Slope	AIC
All Species		830.0	0.58	96.6	0.116	4616.1
Category A	Woody	863.5	0.57	234.8	0.272	870.8
	Herbaceous	590.0	0.83	13.9	0.024	2612.7
Category B	Forb	549.5	0.81	14.0	0.026	1531.0
	Grass	649.9	0.87	13.1	0.020	1073.7
	Shrub	863.5	0.57	234.8	0.272	870.8
Category C	Forb	594.5	0.68	12.0	0.020	1018.3
	Sub-shrub	537.4	0.87	17.7	0.033	502.2
	Leafy shrub	528.1	0.76	116.7	0.221	387.5
	Sclerophyllous shrub	2106.3	0.94	111.3	0.053	396.8
	Long life grass	650.4	0.88	17.0	0.026	616.1
	Short life grass	606.7	0.42	6.1	0.010	403.5
Species specific	*Maireana pyramidata*	2076.4	0.96	113.4	0.055	201.7
	Atriplex vesicaria	726.5	0.95	10.1	0.014	161.4
	Sclerolaena ventricosa	964.3	0.90	16.9	0.018	140.9
	Ptilotus sessilifolius	390.5	0.81	4.3	0.011	182.6
	Abutilon halophilim	500.5	0.86	27.6	0.055	156.5
	Astrebla lappaceae & *A. pectinata*	661.9	0.88	21.4	0.032	371.8
	Thyridolepis mitchelliana	2350.5	0.54	5.0	0.002	191.7
	Eragrostis setifolia	520.1	0.84	6.8	0.013	211.0
	Maireana sedifolia	2360.7	0.80	106.2	0.045	199.6
	Sida petrophila	386.2	0.92	3.2	0.008	113.2
	Austrostipa scabra	566.9	0.84	3.6	0.006	65.0
	Sclerolaena eriacantha	646.2	0.90	3.6	0.006	114.3
	Eremophila gilesii	528.1	0.76	116.7	0.221	387.5
	Rhodanthe floribunda	382.4	0.81	4.0	0.010	150.4
	Calotis lappulacea	228.0	0.57	6.0	0.026	203.3

Table 0.4. Slopes, adjusted R^2, root square mean errors (RMSE), the RMSE/slope ratio and Akaike's information criteria (AIC) for categories and species tested for *cover·height index*-AGB relationships. All relationships are highly significant ($P < 0.001$).

Category/Binomial		Slope (g·m^3)	Adjusted R^2	RMSE (g·m^3)	RMSE/Slope	AIC
All Species		1556.7	0.60	94.0	0.060	4594.8
Category A	Woody	1562.0	0.59	229.9	0.147	868.1
	Herbaceous	1472.3	0.81	14.6	0.010	2646.1
Category B	Forb	1740.2	0.79	14.7	0.008	1547.0
	Grass	1314.0	0.87	13.0	0.010	1072.1
	Shrub	1562.0	0.59	229.9	0.147	868.1
Category C	Forb	2003.8	0.70	11.5	0.006	1008.0
	Sub-shrub	1675.1	0.84	19.6	0.012	514.0
	Leafy shrub	983.7	0.84	94.3	0.96	374.3
	Sclerophyllous shrub	3831.5	0.93	115.0	0.030	398.9
	Long life grass	1310.7	0.88	16.7	0.013	613.6
	Short life grass	2365.2	0.38	6.2	0.003	407.1
Species specific	*Maireana pyramidata*	3696.5	0.98	89.0	0.024	194.0
	Atriplex vesicaria	2360.8	0.97	7.0	0.003	146.0
	Sclerolaena ventricosa	3174.5	0.95	11.5	0.004	128.4
	Ptilotus sessilifolius	1023.1	0.80	4.4	0.004	183.9
	Abutilon halophilim	1659.0	0.85	28.5	0.017	157.5
	Astrebla lappaceae & A. pectinata	1301.2	0.89	20.5	0.016	368.4
	Thyridolepis mitchelliana	8873.0	0.41	5.6	0.001	199.5
	Eragrostis setifolia	1627.7	0.72	8.9	0.005	228.0
	Maireana sedifolia	6553.3	0.84	95.9	0.015	196.3
	Sida petrophila	727.5	0.89	3.8	0.005	120.6
	Austrostipa scabra	2134.8	0.82	3.8	0.002	65.8
	Sclerolaena eriacantha	2944.5	0.86	4.3	0.001	154.5
	Eremophila gilesii	983.7	0.84	94.3	0.096	374.3
	Rhodanthe floribunda	1410.6	0.75	4.6	0.003	157.7
	Calotis lappulacea	805.3	0.53	6.3	0.008	206.7

Within category B, the relationship between AGB and *cover* indicated that the greatest AGB·m^2 was for *shrubs* (863.5 g·m^2) and the lowest AGB·m^2 was for *forbs* (549.5 g·m^2). The *cover·height index* indicated that *grasses* had the lowest AGB·m^3 (1314.0 g·m^3) and *forbs* had the highest (1740.2 g·m^3).

Cover only data for category C groupings resulted in five of the six plant types having AGB/area slopes between 528.1 and 650.4 g·m^2. The exception, *Sclerophyllous shrubs*, had greater than 3 times the AGB for the same area. Using *cover·height index* gave similar results, in that the slope for *sclerophyllous shrubs* was 1466.3 g·m^3 greater than the second large group in category C (2365.2 g·m^3 for *short life grasses*) and even greater than the remaining groups; however, there was noticeably greater range in values compared to *cover*-based estimates overall.

4.3.3 Accuracy of Allometric Estimates in Validation Plots

Standing biomass (g·m^2) for each site is listed in **Table 4.1** and *cover* based estimates are compared in **Figure 4.2** including the percent error of each allometric estimate relative to the validation plot. Using *cover*, all multispecies allometric estimates over-predicted the actual standing biomass. In Charleville and Quilpie, the estimates using *cover* were nearly double that for all multispecies models (**Figure 4.2**). The exception was in Charleville where category C over-predicted biomass by 49.9%; however, the *leafy shrub* group was derived using *E. gilesii* only which is the primary species at this site. Using *cover*, estimates were similarly inaccurate for Cobar, Broken Hill and Milparinka (**Figure 4.2**). *Cover·height index* estimates are presented in **Figure 4.3**, including relative error of multispecies estimates relative to the validation plot. Overall, *cover*-based estimates were more accurate at Nyngan, Quilpie and Broken Hill. *Cover·height index*-based estimates were marginally more accurate at Charleville. Estimates of biomass at Cobar using *cover·height index* were similar (range

from -56.8 to -41.2% error) while *cover* estimates were more variable (range from 28.1 to 92.3% error). Estimates for Milparinka were the most accurate for both *cover* (range from 28.7 to 66.9% error) and *cover·height index* (range from 17.5 to 30.5% error).

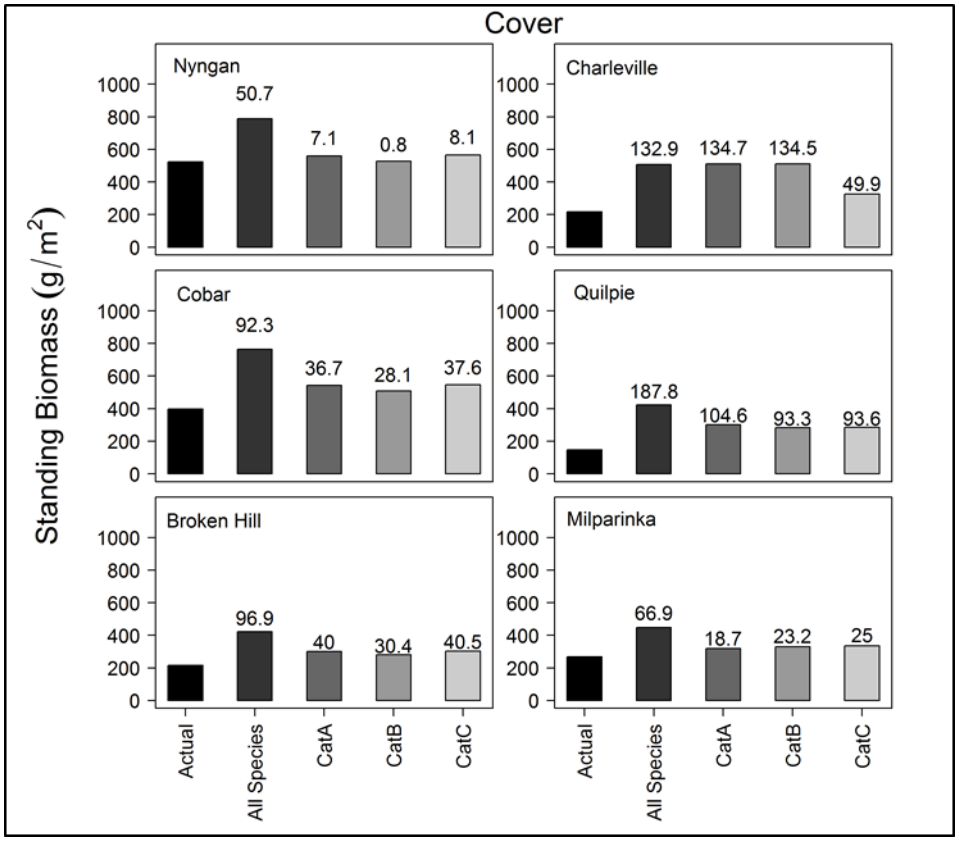

Figure 4.2. Standing biomass from calibration plots (labeled 'Actual' on x-axis) by site. *Cover*-based estimates of standing biomass for multispecies allometric models (all species, category A, category B, and category C) are included. Numbers above bars represent the percent error of the estimate relative to the actual amount of standing biomass from the calibration plot.

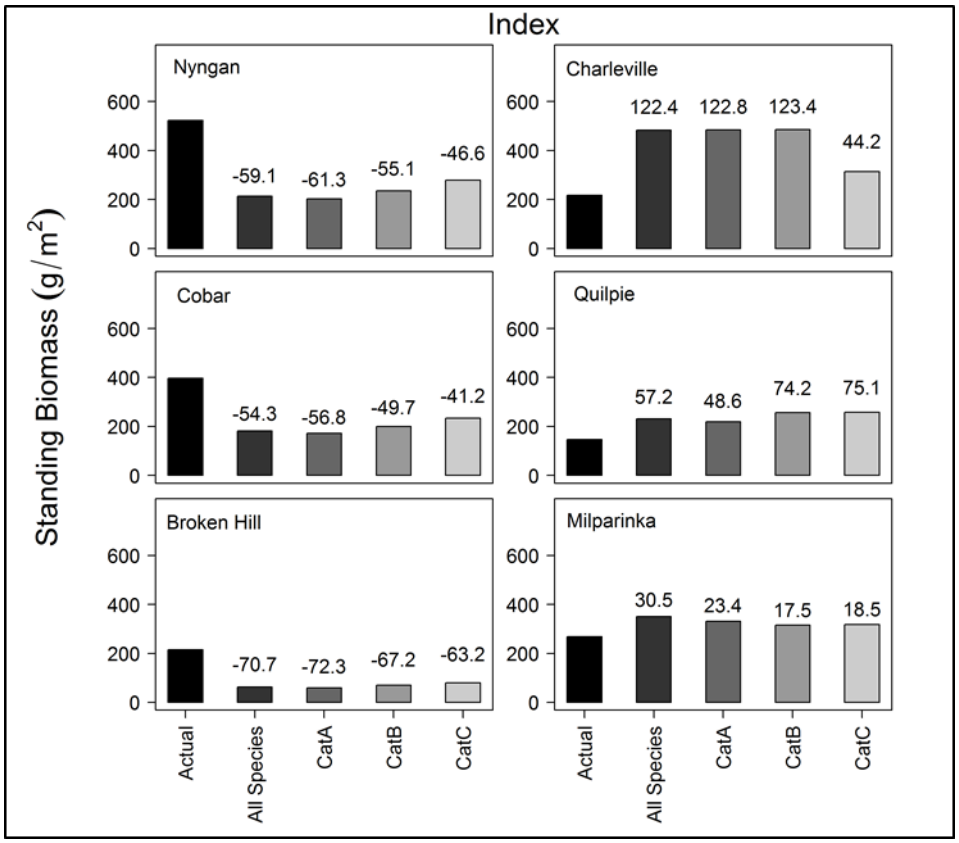

Figure 4.3. Standing biomass from calibration plots in (labeled 'Actual' on x-axis) by site. *Cover·height index*-based estimates of standing biomass for multispecies allometric models (all species, category A, category B, and category C) are included. Numbers above bars represent the percent error of the estimate relative to the actual amount of standing biomass from the calibration plot.

4.3.2 Model Fit Improvement with the Addition of Height

The change in adjusted R^2, AIC, and RMSE are reported in **Table 4.5**. A positive change in adjusted R^2 indicates a model improvement with the addition of height. A positive change in RMSE indicates the error term increased with the addition of height, indicating a less robust model fit. Using *cover·height index* rather than *cover* improved regression equations in four out of twelve instances; however, there were two cases where the addition of height reduced

1424 the model fit. Changes in adjusted R^2, AIC and RMSE were independent of one another. For
1425 example, the largest improvement in adjusted R^2 (11.0%) and RMSE (-19.3%) occurred in
1426 category C *leafy shrubs* while the largest improvement in AIC (21.3 unit-less) occurred when
1427 adding height to the 'all species' estimate. Adding height had the least effect on category C
1428 *sub-shrubs* based on adjusted R^2 and RMSE, and on category A *herbaceous* plants based on
1429 AIC.
1430

Table 0.5. Changes in Adjusted R^2, root square mean error (RMSE) and Akaike's information criteria (AIC) when using *cover·height index* to predict aboveground biomass (AGB), compared to use of cover data alone.

Category	Type	Adjusted R^2 (% Change)	RMSE[1] (% Change)	AIC (Raw Improvement)
All species	All species	3.9	-2.7	21.3[$]
Category A	Woody	3.1	-2.1	2.6
	Herbaceous	-2.2	5.3	-33.4[*]
Category B	Forb	-2.1	4.4	16.0[$]
	Grass	0.2	-0.6	1.6
	Shrub	3.1	-2.1	2.6
Category C	Forb	3.6	-3.9	10.3[$]
	Sub-shrub	-3.5	10.7	-11.8[*]
	Leafy shrub	11.0	-19.3	13.3[$]
	Sclerophyllous shrub	-0.5	3.4	-2.1
	Long life grass	0.5	-1.7	2.5
	Short life grass	-8.3	2.9	-3.6

[1]Note: a positive percentage for adjusted R^2 indicates more variation was explained with the addition of height, while a positive percentage for RMSE indicates less variation was explained in the model. [$]Denotes where the addition of height significantly improved the model estimate. [*]Denotes where the addition of height significantly worsened the estimate.

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4.4 DISCUSSION

In this study, we sought to 1) test whether allometric estimates of AGB using *cover* can be improved by adding height measurements to generate a *cover·height index*; 2) determine if equations based on data from multiple plant species, covering different life histories, were suitable for estimating AGB, and address the level of specificity required for species groupings for accurate estimation of AGB; and 3) determine if landscape-scale estimates derived from multi-site data were suitable for use at the site level, for six sites in eastern Australian rangelands. We found that the addition of height to *cover* (i.e. *cover·height index*) improved allometric estimates in several groups, but reduced the accuracy of estimates for others. As plant categories increased in specificity, we observed an increase in adjusted R^2 values and the ratio of RMSE to the slope, indicating that more specific groupings were better at estimating AGB compared to multispecies estimates (**Tables 4.3** and **4.4**). Finally, we found that the use of generic equations developed using data from multiple sites (at the landscape scale) remains problematic for site-specific biomass estimates, even when using broad functional categories of plants based on life-history and morphological features.

When comparing allometric equations on the basis of adjusted R^2, seven of the twelve categories tested were improved (0.2 to 11.0% improvement) by the addition of height (i.e. using the *cover·height index*), compared to *cover* alone, while the remaining five were less accurate (-8.3 to -0.5% change). Using RMSE we found similar results; estimates that improved when height was added performed in much the same way using both RMSE and R^2 metrics. Based on AIC selection criteria, four groups were significantly improved with the *cover·height index*, while only two groups were negatively affected. We conclude that the inclusion of height to *cover*-based estimates of AGB generally yields more accurate estimates, particularly when grouping species at more specific levels. This provides support for our hypothesis that the inclusion of height may be beneficial for cross-site usage.

The increased amount of data variance explained (adjusted R^2) and reduced error terms (RMSE) with increasing specificity of plant grouping indicates that species-specific estimates of AGB may be better than multispecies estimates contrary to previous studies (Roxburgh et al. 2015; Chojnacky et al. 2013; Paul et al. 2016), at least in herbaceous (Nafus et al. 2009; Flombaum and Sala 2007) and shrub-dominated plant communities (Buech and Rugg 1989; 1995; Mosseler et al. 2014). Therefore, we found evidence that suggests greater specificity of plant groups will yield improved allometric estimates of AGB when using both *cover* and *cover·height index* at these sites. This remains true if we look at the species-specific data (**Tables 4.3** and **4.4**), where adjusted R^2 values were greatest, and the ratio of RMSE to the slope was the lowest, for species-specific estimates compared to multispecies estimates. This supports our hypotheses that greater specificity of plant groupings improve allometric biomass estimates.

The estimates of *cover*-AGB relationships (slopes) for our study were similar to other studies using *cover*. Flombaum and Sala (2007) in the Patagonian steppe, found cover-based AGB estimate slopes of 1,225.1 and 596.3 $g \cdot m^2$ for shrubs and grasses, respectively. Our study indicated slopes ranging from 606.7 to 650.4 $g \cdot m^2$ for grasses; however, our shrub estimates were quite different. For our leafy woody shrubs (a subgroup of CatC), solely represented by *E. gilesii*, we found a slope of 528.1 while our two sclerophyllous species (CatC) had slopes of 2106.3 $g \cdot m^2$. Our estimate based on woody categorization (CatA) was closer to that of Flombaum and Sala (2007) at 863.5 $g \cdot m^2$, which was derived using both leafy and sclerophyllous species, but they differed markedly to those from Ónodi et al. (2017) whose slopes for grass *cover*-AGB ranged from 123.7 to 189.9 $g \cdot m^2$, depending on the year of sampling. It is important to note that estimates presented at the $g \cdot m^2$ and $g \cdot m^3$ may be inflated since none of the individuals sampled were actually that size (see **Table 4.2** for range of sizes sampled by species). This likely explains why, at the per m^3 scale, *forbs* had greater

biomass than *shrubs* (see category B in **Table 4.4**). Because multispecies estimates were derived with more samples (i.e. more species) there was generally a greater range of data along the x-axis (*cover* or *cover·height index*), which could be why multispecies estimates were generally more accurate at predicting biomass in the validation plots. A potential limitation in our experiment is that we selected isolated individuals to develop allometric equations. Having a large neighbour could result in changes in how biomass is located on a focal plant (i.e. changes in leaf area) which could alter the allometric relationship (e.g. increase in leaf area at the expense of height or vice versa). To our knowledge, such investigations have not been conducted in grass- or shrub-dominated ecosystems; however, such data collected would prove difficult in remote locations.The addition of height, previously considered to add little robustness to predicting AGB in grasses (Andariese 1986; Assaeed 1997; Guevara, Gonnet, and Estevez 2002; Nafus et al. 2009), improved our estimates in some cases. There has been an increase in interest in why allometric estimates may not be accurate across years and across sites (Frank and McNaughton 1990; Sala et al. 2000; Shaver et al. 2001; Ónodi et al. 2017). We see the addition of height as a possible mechanistic explanation for changes in the proxy-AGB relationship across temporal and spatial scales, particularly in grasslands where shifts from water to light limitation can occur (Knapp 1984). In other words, periods of high water availability may result in greater aboveground growth (to avoid being shaded), which would not be apparent in the *cover*-AGB relationship. The improvement of allometric estimates with height in this study (**Table 4.3**) occurred for 'all species', *forbs* (Cat B and Cat C) and *leafy shrubs* (Cat C); however, the addition of height negatively affected the estimates of AGB in the *herbaceous* group (Cat A). Interspecific differences amongst plant organ size (e.g. leaf size) and structure (e.g. specific leaf area) may limit the use of multispecies estimates, which others have considered in herbaceous systems (Frank and McNaughton 1990). Therefore, integration of other, less-

easily measured, proxies for AGB may be necessary to more accurately predict AGB such as changes in plant tissue density (leaves and stems) that can vary with environmental conditions.

4.5 CONCLUSION

Using fifteen species from six field sites in the eastern Australian arid and semi-arid rangelands we used both *cover* and a *cover·height* index to estimate AGB. We conclude that using a *cover·height index* was marginally superior to *cover* alone in predicting AGB. The addition of height may represent a mechanistic link that allows the use of allometric equations across years given changes in rainfall or other environmental conditions that can alter plant height without changing cover. In addition, we found AGB estimates based on broader groupings of multiple species to be less robust than for plant groupings based on life history and/or structural characteristics, or species-specific data. We conclude that adding a greater number of species, using additional non-destructive proxy measures (e.g. leaf and stem density) for prediction, and adding data across multiple years, are necessary to accurately estimate carbon stocks and ecosystem sensitivity to external drivers of change in the shrub- and herbaceous-dominated plant communities that characterize the eastern Australian rangelands.

Chapter 5: Timing and intensity of soil moisture deficit differentially affect competition amongst three mesic perennial grasses

5.0 ABSTRACT

Grasslands are crucial for carbon sequestration and maintaining grazing enterprises worldwide. It is well established that grassland productivity is strongly regulated by precipitation patterns, and anticipated reductions in rainfall are likely to have strong negative impacts. The timing and intensity of drought will affect interactions between plant species with cascading effects on plant community dynamics and productivity. *Festuca arundinacea*, *Phalaris aquatica* and *Lolium perenne* are three important mesic grasses that underpin pasture systems worldwide, and understanding their responses to drought is crucial for predicting ecosystem functioning in a changing climate. We subjected these species, grown in isolation and in mixture, to three drought treatments to determine how interspecific interactions (competition and facilitation) were affected under contrasting patterns of soil moisture deficit. Drought treatments were based on physiological indicators and comprised: 1) "short", corresponding to the time (from cessation of watering) it took for the first species to fully close its stomata (i.e. zero stomatal conductance, g_s, a surrogate for physiological activity); 2) "prolonged", representing the length of time it took for the final species to reach zero stomatal conductance; and, 3) "repeated", representing water withdrawal until the first species closed its stomata, followed by a re-wetting and a second drought. Within each drought treatment, plants were separated into two groups so that above- and belowground biomass could be harvested before (resistance period) and after (recovery period) re-watering events. The competitive effects of neighbouring species on target species were calculated to determine how competition affected each species' resistance to, and ability to recover from, drought. In *Lolium* and *Phalaris*, the effects of competition were consistent across all drought

treatments and harvesting periods. *Festuca*, however, was less suppressed by inter-specific competition in the repeated drought (log competitive effect $\bar{x} \pm \sigma_{\bar{x}}$, 0.318 ± 0.076), while there was almost no effect of competition on its ANPP in the short-term drought (-0.007 ± 0.072). Additionally, the strength of competitive suppression of *Festuca* was significantly greater before re-watering (0.275 ± 0.103) compared to after re-watering (0.023 ± 0.070). This suggests that 1) competition during reduced water availability differentially affects species that exhibit similar life-history traits (i.e. mesic perennial grasses) and 2) the intensity and timing of droughts will influence the degree to which species experience competition and associated negative productivity responses. Competition was relatively uniform across the three mesic grasses, but *Festuca* is a strong candidate species in multispecies pastures since negative neighbour effects on productivity were lower than those observed for *Lolium* and *Phalaris*, particularly during mild drought periods and during recovery from drought. The use of multispecies pastures may be necessary in the future to mitigate potential negative effects of more severe and frequent drought periods. This research highlights the importance of biotic interactions between plants as drivers of grassland performance, as well as potential management options to mitigate the negative effects of reduced water availability on productivity.

5.1 INTRODUCTION

The potential negative impacts of changes in precipitation for plant communities have been well-documented (Weltzin et al. 2003; Knapp, Ciais and Smith 2017). Reduced water availability typically leads to down-regulation of photosynthesis resulting in reduced productivity at both the individual and community-scale (Huxman et al. 2004). We also know that the negative effects of reduced water availability are species-specific (Zweifel et al. 2009), indicating that some species will be relatively resistant to reductions in precipitation,

while others will be sensitive. While environmental conditions are a primary determinant of plant community composition (Emerson and Gillespie 2008; Kraft et al. 2015), the interactions between neighbouring species can also affect species composition (Thomson et al. 1996; Callaway and Walker 1997). Less is known about how competitive and facultative interactions between co-occurring plant species will change under predicted changes in climate, such as altered rainfall regimes (Brooker 2006). Here, we aim to determine how different drought duration and frequency may affect the competition between three perennial C_3 grass species, commonly used in pastures worldwide.

Typically, ecologists have associated decreasing resource availability with intensified competition (i.e. the stress-gradient hypothesis) (Grime 1979). While seemingly intuitive, support for this concept has been mixed (Callaway and Walker 1997; Butterfield et al. 2016) and the need to address this topic will become increasingly important given predicted and recently observed changes in climate (Grant et al. 2014; Ploughe et al. 2018). The interactive effects of competition and facilitation between plants along resource-availability gradients have been examined extensively (see Maestre et al. 2009 for review), but less is known about how plant-plant interactions shape plant community dynamics in response to global change (Brooker 2006). For example, atmospheric N deposition leads to increased soil N availability, potentially resulting in scenarios where some species become more abundant by more efficiently competing for the additional N than neighbouring species (e.g. Van den Berg et al. 2005). In this case, an increase in resource-availability results in more competitive interactions between co-occurring plant species. Other studies, however, have observed that competitive interactions are likely species-specific and non-uniform across a site given an alteration in stress or resource availability (Dunnett and Grime 1999; Fitter and Fitter 2002). Such plant-plant interactions can be dependent on seasonal characteristics, edaphic factors, successional stage and plant life stage (Hillier 1990; Walker 1993; Bertness and Shumway

1993; Lee and Caporn 1998; Körner 2003). In grasslands, rainfall is the primary driver of plant productivity (Guo et al. 2012; Griffin-Nolan et al. 2018) and therefore is likely a key resource mediating plant-plant interactions (Grant et al. 2014).

Grasslands are important terrestrial sources of biodiversity and carbon sequestration (Suttie et al. 2005; Dass et al. 2018). Anticipated changes in rainfall periodicity (IPCC 2013) are likely to have strong negative effects on the ecosystem services that grasslands provide (Sala and Paruelo 1997; Carlsson et al. 2017). In one study, Grant et al. (2014) found both competitive and facultative effects as a result of differing, species-specific responses to either extreme drought or flooding. Further, a review by Ploughe et al. (2018) highlighted the system-specific attributes that may determine how plant-plant interactions change under drought. For example, if a re-wetting event that only saturates the top layer of soil occurs after a drought, shallow rooted species might have an advantage. Conversely, following a saturating event, where water availability is uniformly high throughout the root-zone soil, factors such as growth rate and reproductive capacity may become more important in driving plant composition and plant-plant interactions. Thus, experiments addressing this topic are not only warranted but, in fact, crucial for predicting how ecosystems will respond to altered rainfall patterns.

The overall objective of this study was to determine how the timing and intensity of drought affected three common pasture species (*Lolium*, *Festuca* and *Phalaris*), and determine if competition altered their responses to the droughts. We grew the three grasses in isolation, and in mixture (of all three species), and subjected them to three different drought treatments. We defined our drought treatments (short, prolonged, repeated) based on physiologically relevant events (closure of stomata), rather than environmental events (e.g. 40 or 100 days without rainfall). We used stomatal conductance (g_s, mmol H_2O m^{-2}) as a surrogate for physiological activity (Jaleel et al. 2009). Each of the three drought treatments

is described in detail in the *Materials and Methods* section but briefly, we then calculated the difference in biomass produced by each species in isolation compared to their growth in mixture to determine if competitive effects were present during the drought (resistance phase) and following a re-watering period (recovery phase). Plants harvested during the drought and following re-watering were compared with well-watered control plants harvested at the midpoint of the two harvest periods (i.e. the resistance and recovery phase harvests). We hypothesized that shorter, less intense droughts would result in less intensive competitive interactions between plants (i.e. less negative effects on biomass production) than a longer, more severe drought. In addition, we hypothesized (across all drought types) that competition would be greatest during the resistance phase and would be alleviated during recovery (i.e. post re-watering).

5.2 MATERIALS AND METHODS

5.2.1 Study Species

The experiment was conducted from March to November 2018 (representing autumn through spring in the Southern Hemisphere). We studied three C_3 perennial grasses that are commonly used as pasture species and are important to the grazing and livestock industry in south eastern Australia (Reed 1996, Ayres et al. 2000). Seed was obtained for *Phalaris aquatica* L. (cultivar: Holdfast GT), *Lolium perenne* L. (cultivar: Kidman), and *Festuca arundinacea* Schreb. (cultivar: Quantum II MaxP) from Heritage seeds (Dandenong South VIC, Australia).

5.2.2 Experimental Site and Design

The experiment was conducted at Western Sydney University's Hawkesbury Campus using the "Large Rainout Shelters" facility (www.westernsydney.edu.au/hie/facilities/rainout_shelters). The facility has six large shelters (12 x 8 m, 7 m height) with rain-sensing automatically retractable roofs and curtains to exclude ambient rainfall (see Dijkstra et al. 2016 for further description). In each shelter, there are eight overhead sprinklers for irrigation. In addition, each shelter has eight time-domain reflectometry (TDR) sensors that record soil moisture content at fifteen minute intervals in the top 15 cm of soil, which were used to monitor soil moisture in the pots.

From December 1st to 7th 2017, 45 litre black polyethylene planter bags (410 mm height, www.planterbags.com.au) were filled with sandy loam soil collected from Menangle, NSW, Australia (Drake et al. 2015; 2017; Zhang et al. 2018) and placed in the shelters. In each half shelter, 16 bags were evenly spaced (for a total of 12 shelter halves, 16 bags in each and thus an overall total of 192 bags). On March 12th 2018, seeds of each species were sown (5 cm depth) into bags either as single species (10 seeds) or mixtures (10 seeds per species). Pots were well-watered until drought treatments commenced on June 20th 2018 (100 days after sowing). Before commencing treatments, seedlings were removed (over the 100 day period) so that either one individual (isolated plants) or one individual of each species (mixtures) remained. Plants were given liquid fertilizer (12:1.4:7 N:P:K) (Seasol's Powerfeed All Purpose, Bayswater, Victoria) for the first 12 weeks of establishment at which time an initial application of slow-release fertilizer (28:0.4:5) added on DATE became effective (Scott's Slow Release Lawn Fertilizer, Bella Vista, NSW). Fertilizer was applied to minimize potential nutrient limitation.

The goal of the experimental design was to measure whether the response to drought was altered when species were grown in competition with other species, compared to their responses in isolation. We used the same pot size for single individuals and mixtures (three

individuals) to create an environment where individuals had minimal resource limitation, while mixtures would encounter competition, specifically for water, during the imposed droughts. This design has been used frequently to study competition in the absence of manipulating pot size or the number of individuals in a single species pot (Newbery and Newman 1978; J. B. Wilson 1988; Gerry and Wilson 1995; Schwinning et al. 2017).

5.2.3 Determination of Drought Treatments

We applied three treatments representing short-term, prolonged and repeated drought (**Figure 5.1**). In all cases, the goal was to impose droughts as physiologically relevant events (i.e. two weeks following stomatal closure), rather than environmental events (e.g. 40 or 100 days without water). Specifically, when a species reached "near-zero" stomatal conductance (g_s), a reference parameter for physiological activity (Medrano et al. 2002), half the replicates of drought plants were harvested to determine how resistant biomass production was to the drought. The remaining plants in that treatment were subsequently watered for several weeks (2 to 4 weeks depending on treatment) to determine their resilience (i.e. recovery) in terms of biomass production. Near-zero g_s was assumed to occur at *ca.* 50 mmol m^{-2} s^{-1} or lower, using the portable leaf porometer (Model SC-1, Decagon Devices, Pullman, WA, USA) in the field. Well-watered control plants were harvested at the midpoint between resistance and recovery harvests, and were specific to each of the three treatments. Specifically, one group of control plants was harvested at the midpoint between resistance and recovery harvests for the "short" drought plants (**Figure 5.1a** and **5.1b**); a second group of control plants was harvested at the midpoint between resistance and recovery harvests for the "prolonged" drought plants (**Figure 5.1a** and **5.1c**); a third group of control plants was harvested at the midpoint between resistance and recovery harvests for the "repeated" drought plants (**Figure 5.1a** and **5.1d**). *Post-hoc* analyses of the total biomass from well-watered/control plants

showed that there was a 2.7% increase in biomass from November 1st to November 12th 2018. Hence, although the control plant harvests were one week removed from the "short" drought plant harvests, and two weeks removed from the "prolonged" and "repeated" drought plant harvests, the impact of this temporal variation was unlikely to affect data interpretation. In addition, there were no significant differences in aboveground biomass, belowground biomass, total biomass, or the root to shoot ratio for control plants harvested on September 13th, November 1st, or November 12th (dates corresponding to each of the drought treatments).

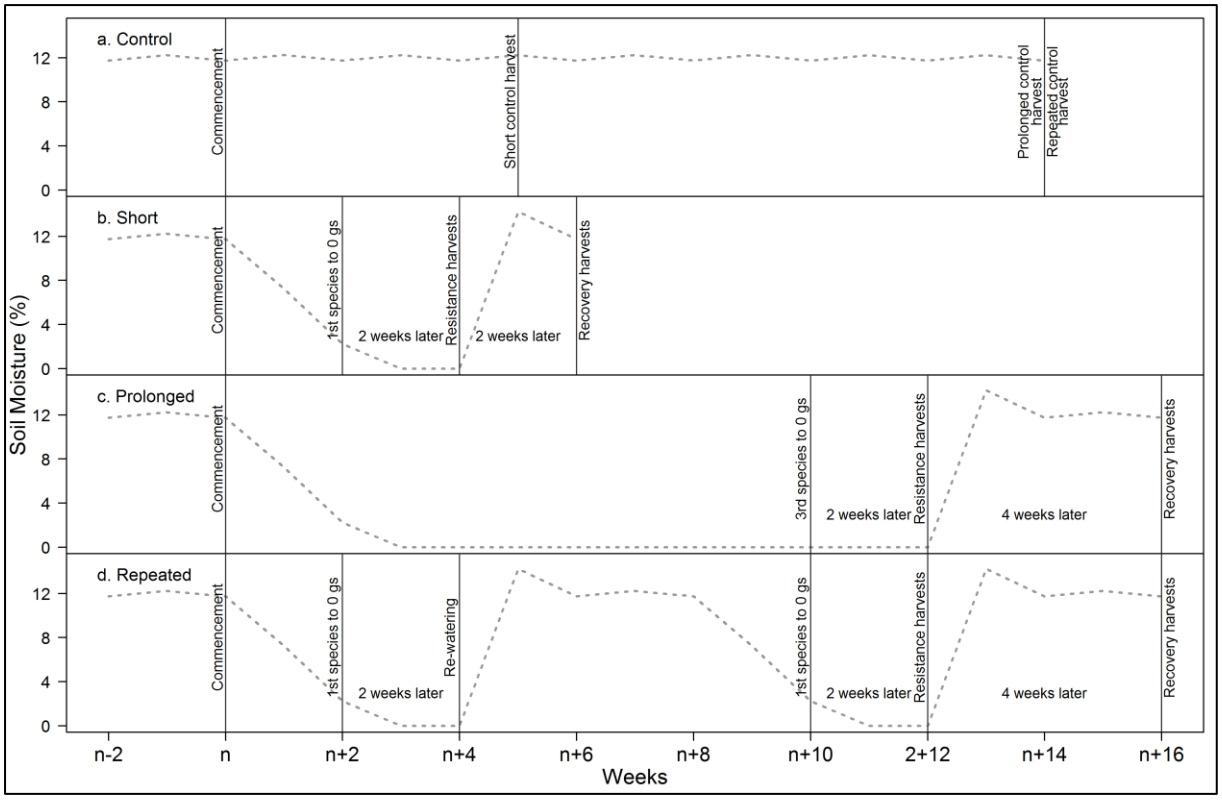

Figure 5.1. Theoretical diagram of the soil moisture (y-axis) treatments over time. Control plants were maintained with regular water inputs to keep soil moisture close to field capacity (panel a). The short drought was determined as the point at which the first species had near-zero g_s for two weeks, at which time plants were harvested for resistance measurements (panel b). Following two weeks of re-watering, plants were harvested for recovery measures. The prolonged drought was similar to the short-term drought; however, it was based around the last (third) species to have near-zero g_s (panel c). The repeated drought was the short-term drought implemented twice with a re-watering period after the first species had near-zero g_s for two weeks (panel d). Control harvests occurred at the midpoint of the resistance and recovery harvests for each of the drought types (panel a).

In the “short” drought treatment, water was withheld until the first of the three species reached near-zero g_s for two weeks. *Lolium perenne* was the first species to experience near-zero g_s for two weeks, after 79 days of withholding watering (**Figure 5.2**). Half the droughted plants were harvested at this time, and the remaining plants were re-watered and subsequently maintained at field capacity. The following week control plants were harvested. The next week the recovered drought plants were harvested.

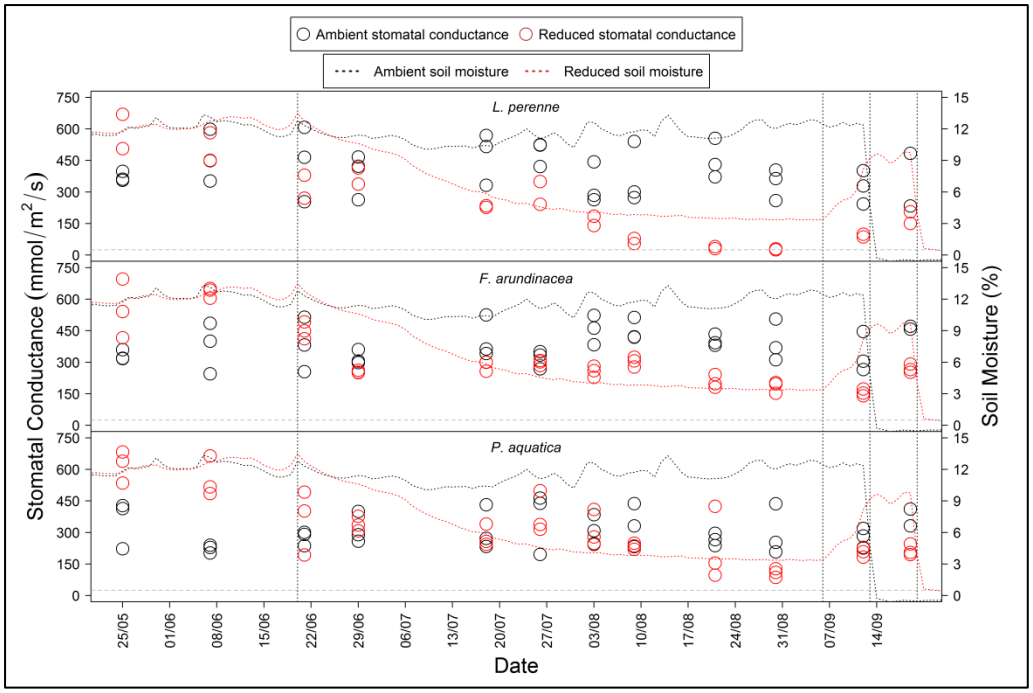

Figure 5.2. Soil moisture (lines, x-right axis) and stomatal conductance (g_s, circles, x-left axis) of *Lolium perenne* (top panel), *Festuca arundinacea* (middle panel), and *Phalaris aquatica* (bottom panel) during the first drought treatment (i.e. short drought). The vertical line at June 20th 2018 represents the first period when water was withheld in drought pots (red). The vertical line at September 6th 2018 represents two weeks of near-zero g_s in the first species (*L. perenne*) at which time half of the plants of all species in this treatment were harvested to calculate drought resistance. The vertical line depicted at September 13th 2018 represents when control plants were harvested. The vertical line at September 20th 2018 represents two weeks of re-watering (following resistance plant harvests) to evaluate drought recovery. Horizontal dashed lines represent the point of near-zero g_s.

In the second treatment, the last species to reach near-zero g_s for two weeks was *F. arundinacea*, which did so after 118 days without watering (**Figure 5.3**). At this time half the plants in this treatment were harvested to determine their resistance to drought, and the remaining plants were re-watered and maintained at field capacity. Two weeks later the designated control plants were harvested. A further two weeks later the remaining plants were harvested to evaluate "recovery".

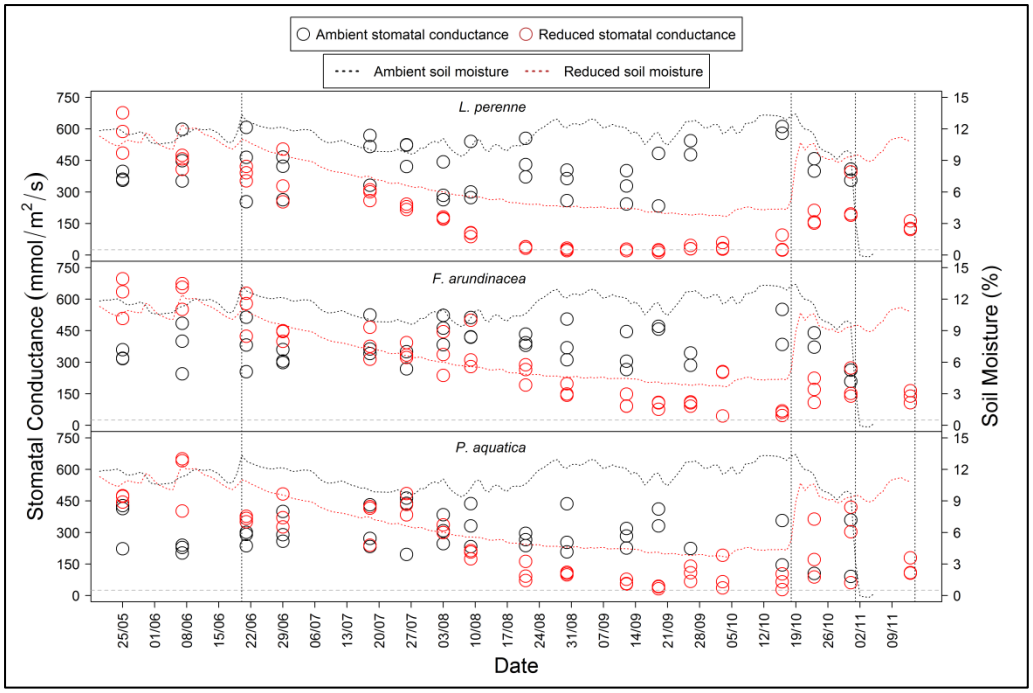

Figure 5.3. Soil moisture (lines, x-right axis) and stomatal conductance (g_s, circles, x-left axis) of *Lolium perenne* (top panel), *Festuca arundinacea* (middle panel) and *Phalaris aquatica* (bottom panel) during the second drought experiment (i.e. prolonged drought). The vertical line at June 20th 2018 represents when the water started being withheld in droughted plants (red). The vertical line at October 17th represents two weeks of near-zero g_s in the third/last species (*F. arundinacea*) at which time plants were harvested to determine their drought-resistance. The vertical line at November 1st 2018 represents when control plants were harvested. The vertical line at November 14th 2018 represents four weeks of re-watering (following resistance plant harvests) to determine drought resilience. Horizontal dashed lines represent near-zero g_s based on leaf porometer data.

In the third treatment, we conducted two consecutive short-term droughts. Water was withheld until the first species reached near-zero g_s (*L. perenne*, after 79 days as described above). At this point, all plants were re-watered to full soil water holding capacity, and a subsequent second dry-down commenced immediately after this was achieved (**Figure 5.4**). After an additional 47 days, both *L. perenne* and *P. aquatica* had reached near-zero g_s. Half the droughted plants were then harvested with the remainder re-watered and maintained at full soil moisture capacity for four weeks, before then being harvested. Designated control plants were harvested at the midpoint of resistance and recovery harvests.

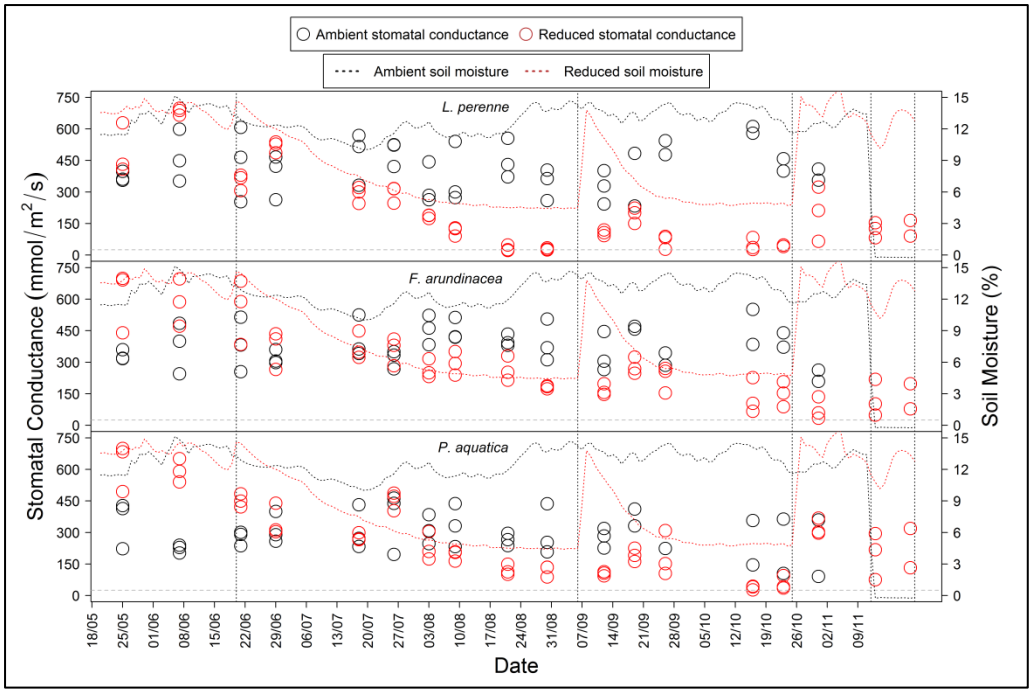

Figure 5.4. Soil moisture (lines, x-right axis) and stomatal conductance (g_s, circles, x-left axis) of *Lolium perenne* (top panel), *Festuca arundinacea* (middle panel) and *Phalaris aquatica* (bottom panel) during the third drought experiment (i.e. repeated drought). The vertical line at June 20th 2018 represents when the water started being withheld in droughted plants (red). The vertical line at September 6th 2018 represents two weeks of near-zero g_s in the first species (*L. perenne*) at which time plants were re-watered to field capacity over 3 days. The vertical line at October 24th 2018 represents two weeks of near-zero g_s in the first species (*L. perenne*) when plants were harvested to quantify their resistance to repeated drought. The vertical line at November 12th 2018 represents the point at which control plants were harvested. The vertical line at November 22nd 2018 represents four weeks of re-watering (following resistance plant harvests) to determine the resilience of plants to two short-term droughts. Horizontal dashed lines represent near-zero g_s based on leaf porometer data.

5.2.4 Harvest Measurements

During each sampling period, above- and below-ground biomass were quantified, the latter following sieving (2 mm) and subsequent washing. It was not possible to separate root biomass to species in the mixtures due to root entanglement. Harvested plant material was dried at 70 °C for ~2-3 weeks prior to subsequent weighing.

5.2.5 Data Analysis

The 6 shelters, each with two separate irrigation zones, resulted in 12 experimental zones. We had 16 planter bags in each zone (12 zones, 16 bags in each) for a total of 192 planters. In order to randomize the treatment combinations into pairs, we used three zones as controls). Each drought treatment was replicated three times (3 droughts and 3 replicates, for a total of 9 zones) and paired with a unique drought. During harvests, half of the plants from each drought treatment were used to estimate drought resistance (plants harvested before re-watering) with the remainder used to estimate the drought recovery (plants harvested two/four weeks after re-watering). For each zone, four soil moisture sensors were available and installed in pots of one of each species/mixture (n = 48 sensors). Of these plants, isolated individuals were monitored for g_s due to time constraints (n = 36).

We examined the response of aboveground biomass, belowground biomass, total biomass and the ratio of root to shoot biomass (R:S) for each drought. Within each drought type we compared plant biomass during the respective drought (i.e. resistance) and recovery period (i.e. resilience) with plants grown in well-watered conditions. In addition, we wanted to determine if the duration of drought and sampling period (before or after re-watering) led to changes in competition amongst the three grasses. To quantify the competitive effects of neighboring species on target species we calculated the log ratios of plants grown alone

versus in mixture (**Equation 5.1**), using only aboveground biomass since root biomass could not be separated in mixtures, as has also been observed by others (Schwinning et al. 2017; Wang et al. 2018). The competitive effects, therefore, of neighboring species on target species were calculated as:

$$\textbf{Equation 5.1}: \log CE_X = \log\left(\overline{B_X}\right) - \log(B_{X,N})$$

where *X* is the species index for the target species and *N* stands for the neighbors of the target species (Goldberg et al. 1999; Schwinning et al. 2017). $\overline{B_X}$ is the average biomass value for species *X* grown in isolation and $B_{X,N}$ is the biomass of the focal plant, *X*, growing with neighbors (*N*) that match drought duration (i.e. short, prolonged, or repeated) and harvesting period (i.e. resistance or recovery). For clarity, the less biomass produced by a target species when grown in mixture ($B_{X,N}$), the greater the competitive effect (CE_X). Conversely, if the biomass produced by a target species in competition ($B_{X,N}$) is greater when compared to biomass produced when in grown in isolation (B_X), the competitive effect (CE_X) would be negative (effectively indicating facilitation).

All data were analysed in R (V3.2.5, R Core Team 2013) using linear mixed models (*lme4* package). Drought treatment type (i.e. short, prolonged or repeated drought), harvesting period (i.e. resistance, control, recovery) and the presence of a neighbor (binary) were used as fixed effects while the shelter in which the pots were place was specified as a random effect. Residual degrees of freedom were estimated using Kenward-Roger Degrees of Freedom Approximation (*LMERTEST* package) (Kenward and Roger 1997). Post-hoc Tukey 'single-step' comparison was employed when fixed effects resulted in significance ($\alpha = 0.05$) (*multcomp* package) (Newell and Douglas 2014; Bretz et al. 2016; Hothorn et al. 2017). Data were log transformed when necessary to ensure homogeneity of variance, which was tested with Levene's test (Katz et al. 2009) in the *car* package. The regulation of g_s by soil moisture

was analysed using linear regression analysis. Normality of residuals was tested using Shapiro-Wilks test (Razali and Wah 2011). Alpha was set at 0.05.

5.3 RESULTS

5.3.1 Above and Belowground Biomass Dynamics in response to Short Drought

Aboveground biomass (AGB) in *Lolium* was unaffected by harvesting period (P = 0.069) or the presence of neighbors (P = 0.407) during the "short drought" (**Figure 5.5**). When examining the effect of harvesting period alone (i.e. neighbor dropped as a fixed effect in the LMM), we found that AGB was greatest in controls (P = 0.041), while there was no difference in resistance and recovery plant AGB (P = 0.897). Aboveground biomass in *Festuca* was not significantly affected by the presence of neighboring species during the short-term drought (P = 0.631), but was affected by harvesting period (P = 0.002). Specifically, AGB was lowest in plants harvested at the peak of the short drought (i.e. the resistance phase), greatest in un-droughted controls and intermediate in those harvested during the recovery period (P = 0.002). Like *Lolium*, *Phalaris* was unaffected by harvesting period (P = 0.0535) or the presence of neighbours (P = 0.176). When examining the effect of harvesting period alone (i.e. neighbour removed as a fixed effect in the LMM), we found AGB for *Phalaris* was greatest in controls (P < 0.002), while there was no difference in plant AGB during resistance and recovery phases (P = 0.458).

Belowground biomass (BGB) in *Lolium* was unaffected by harvesting period (P = 0.103). In *Festuca*, BGB was lowest in plants harvested at the peak of the short drought (i.e. resistance harvest) (P = 0.018). There was, however, no evidence of recovery, with BGB not significantly different from the controls. In *Phalaris*, BGB was lowest in droughted plants

(resistance harvest) and greatest in controls, while plants harvested during recovery had intermediate BGB (P = 0.012). The same was true for BGB in mixtures (P = 0.010).

Total biomass (TB, above and belowground combined) in *Lolium* was unaffected by harvesting period (P = 0.580). In *Festuca*, TB was lowest in droughted plants (resistance harvest), but recovered to control levels upon re-watering (P = 0.016). Total biomass for *Phalaris* was lowest in droughted plants and greatest in controls, while recovery plants were intermediate between these two (P = 0.020). In mixtures, TB was not different between plants harvested in the resistance and recovery phases (P = 0.728) which had significantly less TB compared to control plants (P < 0.001). The ratio of aboveground biomass to belowground biomass (i.e. root to shoot ratio, RS) was unaffected by harvesting period for all individual species and mixtures (**Table 5.1**).

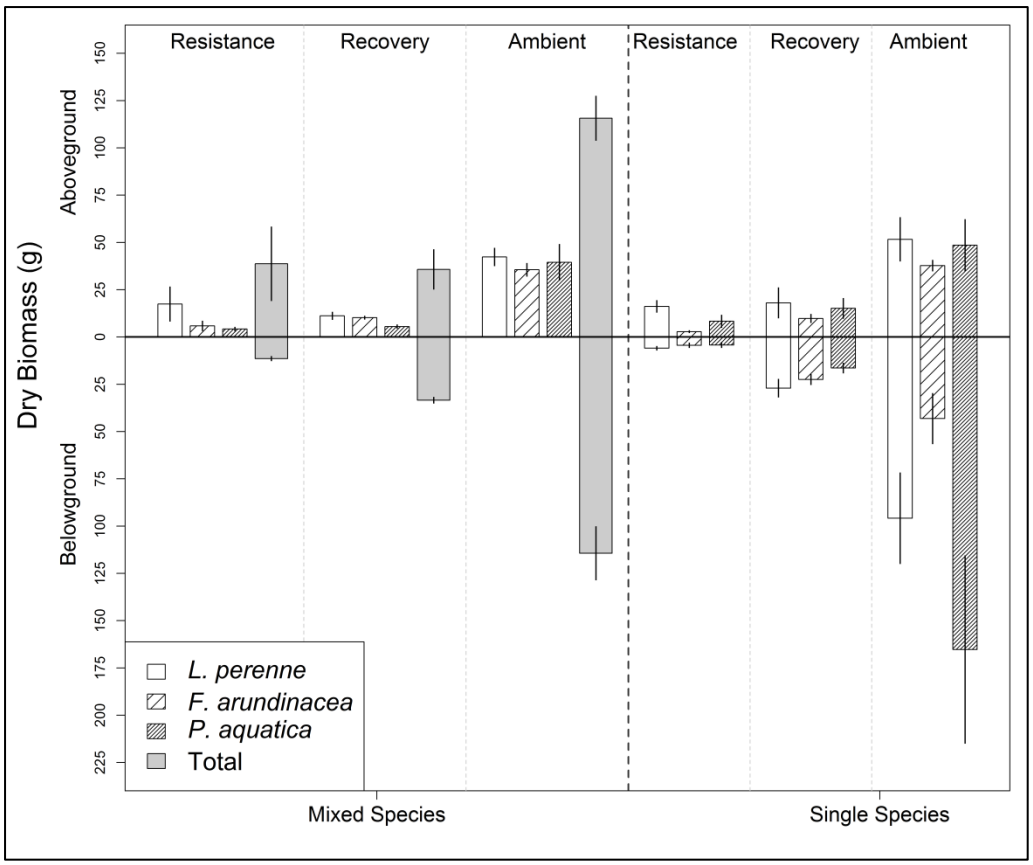

Figure 5.5. Above and belowground dry biomass (in grams) for mixtures and individual (single) species, with standard error bars, during the short-term drought.

Table 0.1. Resulting F statistics and *P*-values from linear mixed model and Kenward-Roger Degrees of Freedom Approximation from the short-term drought experiment. The effect of harvesting period (resistance, recovery, control) is denoted as RRC (degrees of freedom = 2). The effect of neighbor presence is denoted as N (degrees of freedom = 1). "RRC*N" indicates the interactive effect. Significant treatment effects are denoted with asterisks.

Metric	Species	RRC		Neighbors		RRC*N	
		F stat	*P*-value	F stat	*P*-value	F stat	*P*-value
Aboveground	*Lolium*	9.61	0.041*	0.55	0.469	0.27	0.768
	Festuca	36.29	0.002*	0.24	0.631	1.66	0.212
	Phalaris	8.35	0.035*	1.99	0.176	0.32	0.729
Belowground	*Lolium*	6.09	0.103	NA	NA	NA	NA
	Festuca	15.53	0.018*	NA	NA	NA	NA
	Phalaris	15.57	0.012*	NA	NA	NA	NA
	Mixture	26.56	0.010*	NA	NA	NA	NA
Total	*Lolium*	0.63	0.580	NA	NA	NA	NA
	Festuca	16.62	0.016*	NA	NA	NA	NA
	Phalaris	9.62	0.020*	NA	NA	NA	NA
	Mixture	15.26	0.033*	NA	NA	NA	NA
Root/Shoot	*Lolium*	1.85	0.315	NA	NA	NA	NA
	Festuca	2.21	0.243	NA	NA	NA	NA
	Phalaris	2.87	0.127	NA	NA	NA	NA
	Mixture	2.60	0.234	NA	NA	NA	NA

5.3.2 Prolonged Drought Aboveground and Belowground Biomass Dynamics

For the prolonged drought, AGB (**Figure 5.6**) in *Lolium* was unaffected by harvesting period ($P = 0.067$); however, AGB was significantly greater for plants grown in isolation compared to *Lolium* plants in mixture ($P = 0.013$, **Table 5.2**). AGB of *Festuca* was affected by harvesting period ($P = 0.003$), the presence of neighbours ($P < 0.001$), and the interaction between the two ($P = 0.014$). *Festuca* plants grown individually had the lowest AGB during the resistance phase, but recovered to AGB observed in the controls upon recovery. The same was true for *Festuca* when grown in mixture. *Post-hoc* analysis indicated the significant interaction between harvest period and the presence of neighbours resulted from greater AGB production of control *Festuca* plants when grown in isolation compared to those in mixtures ($P < 0.001$). AGB of *Phalaris* was significantly affected by harvesting period ($P = 0.032$), but not by the presence of neighbours ($P = 0.071$) nor their interaction ($P = 0.892$). For *Phalaris*, there was no difference between AGB harvested during the resistance and recovery phases ($P = 0.923$), but both were significantly less than control plants ($P < 0.001$). Overall, AGB was greater in individual *Phalaris* plants compared to those grown in mixture ($P = 0.037$).

In isolation, BGB of *Lolium* was unaffected by harvesting period ($P = 0.121$). BGB of *Festuca* was lowest in the plants during the resistance phase and greatest in controls, but intermediate upon recovery ($P = 0.006$). Belowground biomass for *Phalaris* was lowest in plants during the resistance phase, but recovered to control levels upon re-watering ($P = 0.031$). In mixture, BGB was lowest in plants during the resistance phase and greatest in controls, but intermediate upon recovery ($P = 0.018$).

Total biomass for *Lolium* grown on its own was similar between resistance and recovery harvests ($P = 0.776$), which were significantly less than the corresponding controls ($P = 0.038$). In isolated *Festuca*, TB was lowest in plants in the resistance phase and greatest in controls, but intermediate upon recovery ($P = 0.012$). The same was true for the TB of

1924 mixtures (P = 0.013). Isolated *Phalaris*'s TB upon recovery was comparable to controls (P =
1925 0.447), which were both significantly greater than TB during the resistance phase (P =
1926 0.047).
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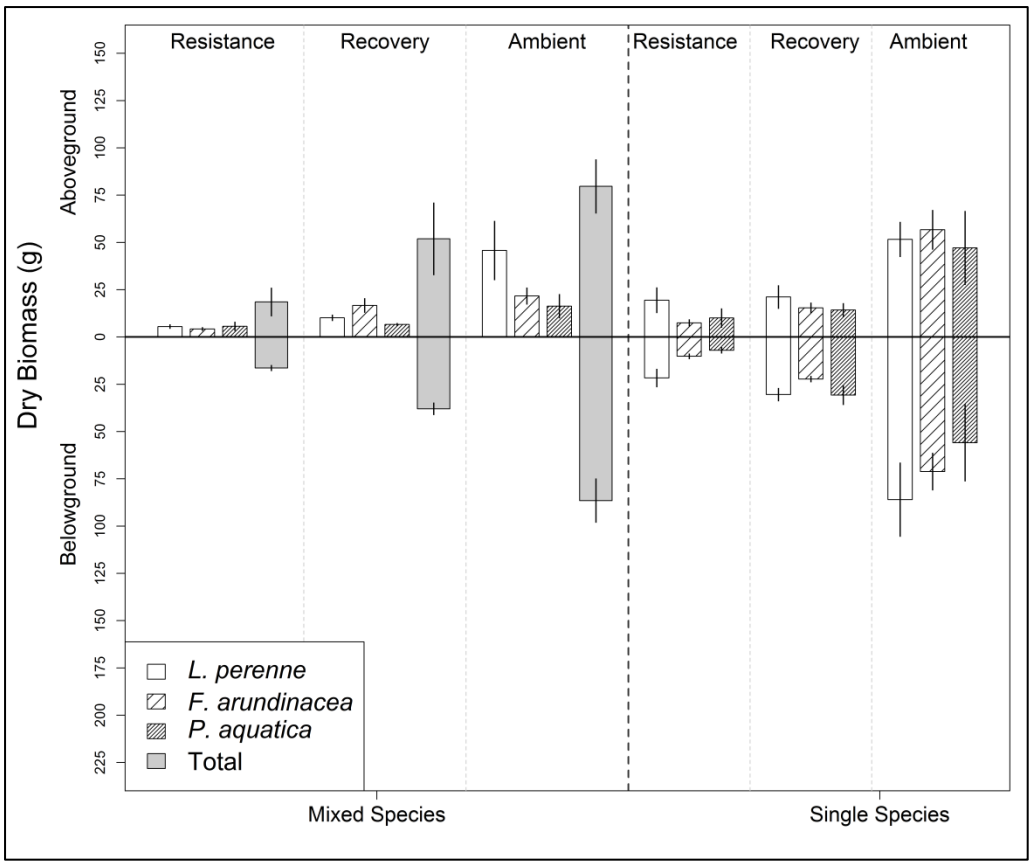

Figure 5.6. Above and belowground dry biomass (in grams) for mixtures and isolated (single) species, with standard error bars, during the prolonged drought.

Table 0.2. Resulting F statistics and *P*-values from linear mixed model and Kenward-Roger Degrees of Freedom Approximation from the prolonged drought experiment. The effect of harvesting period (resistance, recovery, control) is denoted as RRC (degrees of freedom = 2). The effect of neighbor presence is denoted as N (degrees of freedom = 1). "RRC*N" indicates the interactive effect. Significant treatment effects are denoted with asterisks.

Metric	Species	RRC		Neighbours		RRC*N	
		F stat	*P*-value	F stat	*P*-value	F stat	*P*-value
Aboveground	*Lolium*	5.57	0.067	7.31	0.013*	0.62	0.549
	Festuca	29.04	0.003*	16.28	< 0.001*	5.18	0.014*
	Phalaris	7.55	0.032*	3.58	0.071	0.12	0.892
Belowground	*Lolium*	3.88	0.121	NA	NA	NA	NA
	Festuca	46.62	0.006*	NA	NA	NA	NA
	Phalaris	14.94	0.031*	NA	NA	NA	NA
	Mixture	13.42	0.018*	NA	NA	NA	NA
Total	*Lolium*	10.19	0.038*	NA	NA	NA	NA
	Festuca	25.37	0.012*	NA	NA	NA	NA
	Phalaris	8.27	0.047*	NA	NA	NA	NA
	Mixture	16.74	0.013*	NA	NA	NA	NA
Root/Shoot	*Lolium*	0.26	0.810	NA	NA	NA	NA
	Festuca	0.24	0.796	NA	NA	NA	NA
	Phalaris	0.75	0.539	NA	NA	NA	NA
	Mixture	0.18	0.842	NA	NA	NA	NA

5.3.3 Drought Aboveground and Belowground Biomass Dynamics

During the repeated drought treatment (**Figure 5.7**, **Table 5.3**), both harvesting period and the presence of neighbours affected AGB of *Lolium*, while there was no significant interaction between the two treatments. *Lolium* had the lowest AGB during the resistance phase while plants had AGB comparable to controls ($P < 0.001$) at the recovery harvest. There was no significant effect of neighbour presence on *Lolium* (i.e. harvest period dropped as a fixed term from LMM). For *Festuca*, AGB was only affected by harvest period, with significantly lower biomass recorded for the repeatedly droughted plants, compared to both the control plants and those allowed to recover prior to harvesting ($P = 0.005$). AGB in *Phalaris* was lowest in plants during the resistance phase, but recovered upon re-watering ($P < 0.001$). Overall, *Phalaris* had greater AGB when in isolation compared to when grown in mixture ($P = 0.031$). There were no interactive effects of harvest period and presence of neighbours on *Phalaris*'s AGB ($P = 0.523$).

In isolated *Lolium*, BGB was lowest in plants during resistance and greatest in controls, with BGB following recovery as intermediate between these two ($P < 0.001$). Isolated *Festuca* during the resistance phase had the lowest BGB which recovered to control levels upon re-watering ($P = 0.008$). The same was true for BGB in isolated *Phalaris* plants ($P < 0.001$) and BGB in mixtures ($P < 0.001$).

In isolated *Lolium*, TB was lowest during the resistance phase and greatest in controls, with BGB in re-watered (recovery) plants intermediate between the two ($P < 0.001$). Total biomass of isolated *Festuca*, isolated *Phalaris* and mixtures were lowest during the resistance phase, but recovered to control amounts upon re-watering ($P = 0.025$, $P < 0.001$, and $P < 0.001$, respectively). The RS ratios of *Lolium* and *Phalaris* were unaffected by harvesting period ($P = 0.210$). For *Festuca* and mixtures, the RS was not significantly different between controls and the resistance phase ($P = 0.999$ and $P = 0.297$, respectively), but was

significantly greater in re-watered plants following recovery ($P < 0.047$ and $P < 0.001$, respectively).

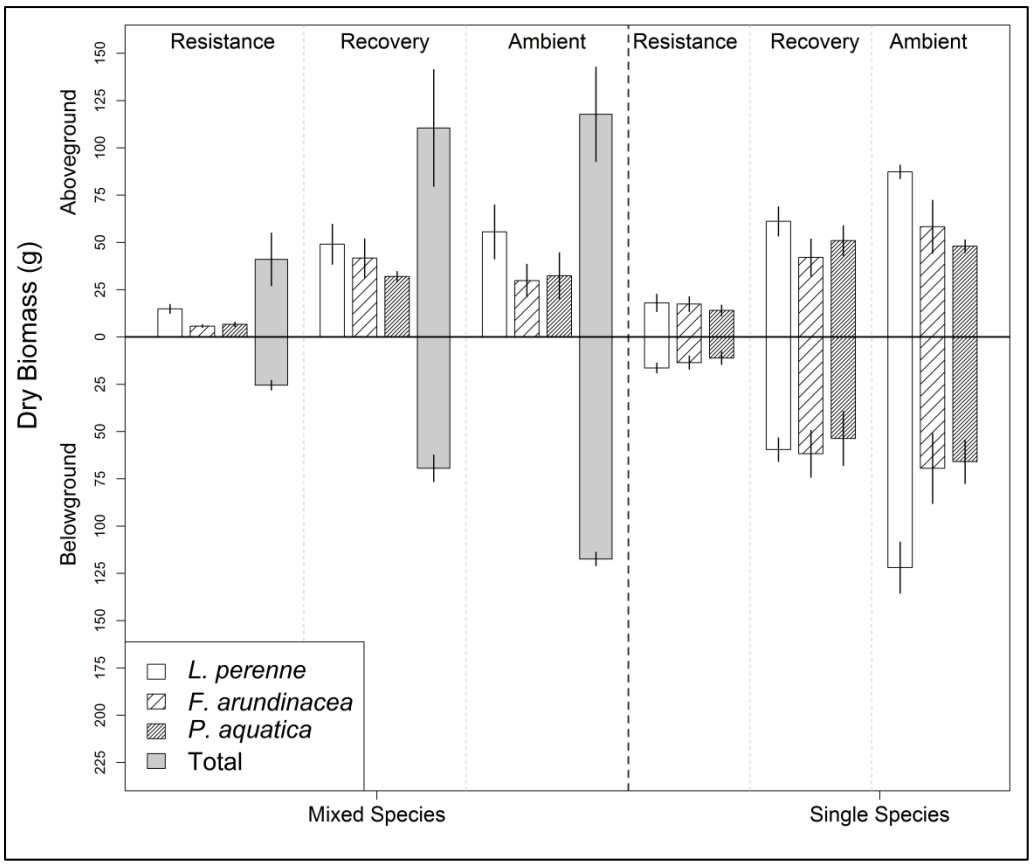

Figure 5.7. Above and belowground dry biomass (in grams) for mixtures and isolated (single) species, with standard error bars, during the repeated drought.

Table 0.3. Resulting F statistics and *P*-values from linear mixed model and Kenward-Roger Degrees of Freedom Approximation from the repeated drought experiment. The effect of harvesting period (Resistance, recovery, control) is denoted as RRC (degrees of freedom = 2). The effect of neighbor presence is denoted as N (degrees of freedom = 1). "RRC*N" indicates the interactive effect. Significant treatment effects are denoted with asterisks.

Metric	Species	RRC		Neighbors		RRC*N	
		F stat	*P*-value	F stat	*P*-value	F stat	*P*-value
Aboveground	*Lolium*	33.86	< 0.001*	1.66	0.208	0.65	0.531
	Festuca	11.01	0.005*	3.88	0.062	2.17	0.138
	Phalaris	67.78	< 0.001*	26.13	< 0.001*	0.67	0.523
Belowground	*Lolium*	41.07	< 0.001*	NA	NA	NA	NA
	Festuca	9.60	0.008*	NA	NA	NA	NA
	Phalaris	23.73	< 0.001*	NA	NA	NA	NA
	Mixture	23.14	< 0.001*	NA	NA	NA	NA
Total	*Lolium*	22.7	< 0.001*	NA	NA	NA	NA
	Festuca	6.79	0.025*	NA	NA	NA	NA
	Phalaris	36.28	< 0.001*	NA	NA	NA	NA
	Mixture	30.79	< 0.001*	NA	NA	NA	NA
Root/Shoot	*Lolium*	1.98	0.210	NA	NA	NA	NA
	Festuca	5.44	0.019*	NA	NA	NA	NA
	Phalaris	1.73	0.241	NA	NA	NA	NA
	Mixture	8.36	0.006*	NA	NA	NA	NA

5.3.4 Drought Type and Recovery Effects on Competition

In *Festuca*, harvest period and drought type had significant effects on competition with no significant interactive effect (**Table 5.4**). Specifically, competition from neighbouring species had bigger effects on *Festuca*'s AGB during resistance and control periods; however, competition significantly declined upon re-watering (**Figure 5.8a**). In addition, the duration and severity of drought affected competitive effects on *Festuca* (**Figure 5.8b**). During the short drought, competitive effects on *Festuca* was near neutral indicating slight competition or facilitation by neighbouring species; in contrast, stronger effects of competition on Festuca were apparent in the repeated drought. The performance of *Phalaris* and *Lolium* were unaffected by competition with neighbouring species (**Table 5.4, Figure 5.8**).

Table 0.4. Resulting F-values and *P*-value from linear mixed models examining the effects of harvesting period, drought type, and their interaction, on species-specific competition. Significant effects are denoted with asterisks.

Species	Harvesting Period (HP)		Drought Type (DT)		HP * DT	
	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
F. arundinacea	8.57	< 0.001*	3.83	0.033*	2.09	0.104
P. aquatica	0.99	0.384	0.56	0.574	0.48	0.752
L. perenne	1.14	0.331	1.48	0.241	0.73	0.569

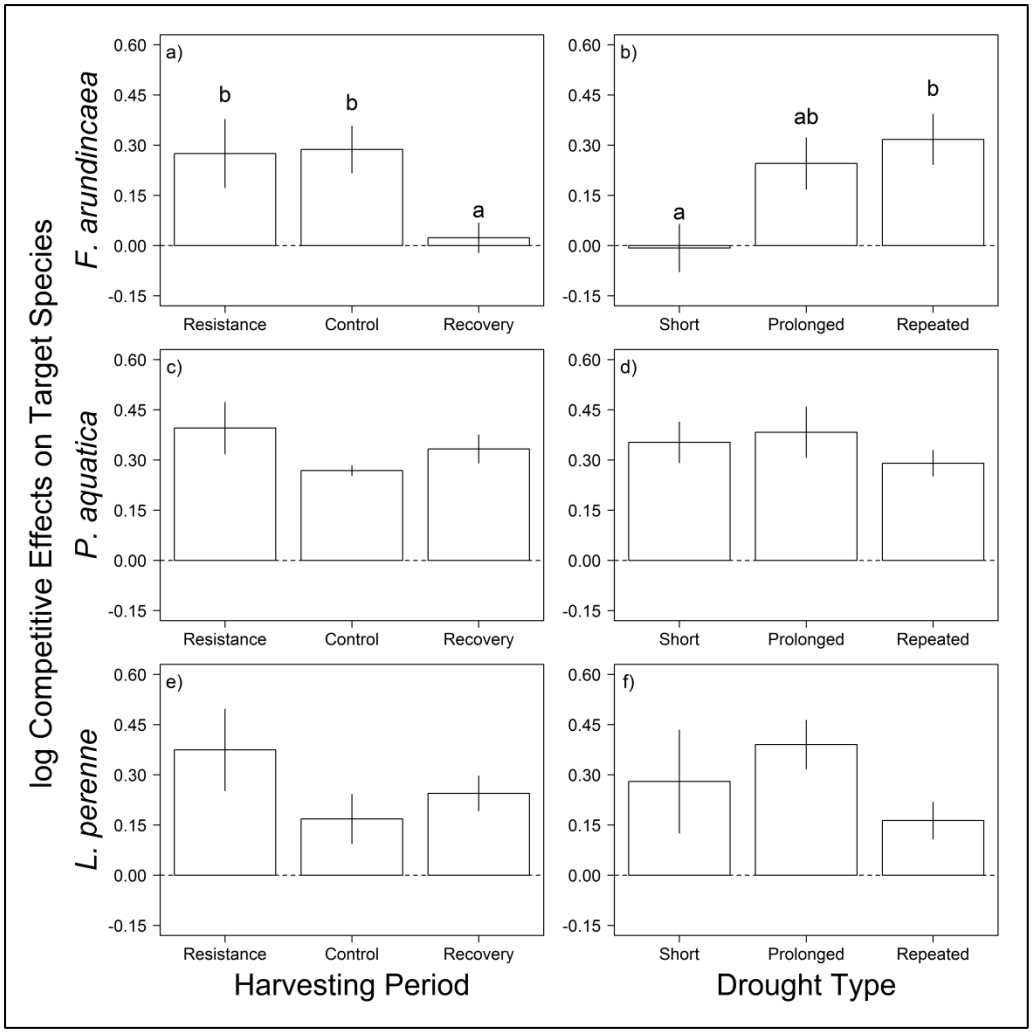

Figure 5.8. Species-specific effects of competition (mean ± standard error) for harvest period (panels a, c, and e) and drought type (panels b, d, and f). Greater competitive effects indicate a negative aboveground biomass response as a result of competition (i.e. when growing with the other species, compared to when grown alone). Different letters above bars within a panel represent significant differences from Tukey single-step post-hoc analysis at 95% confidence. Panels with no letters indicate no effect of harvesting period or drought type on competitive effects.

5.3.5 Stomatal Conductance and Soil Moisture Relationships

The overall linear relationship between soil moisture and g_s was significant for all three species, across all treatments (**Table 5.5**, **Figure 5.9**). This was also the case for the prolonged and repeated droughts, although in the short drought, there was no significant relationship between soil moisture and g_s in *Phalaris*. In general, *Festuca* had slopes that were lower than the remaining species (**Table 5.5**) indicating minimal change in g_s given declines in soil moisture. *Festuca* was also the last species to reach near-zero g_s in the prolonged drought treatment. *Phalaris* had the greatest slopes across all treatments and overall, indicating that it was particularly responsive to changes in soil moisture. In the prolonged drought, *Lolium* had the lowest slope. This species was, however, observed to cease g_s more rapidly than other species resulting in the accumulation of data where g_s is near-zero across a range of soil moisture values (**Figure 5.9b**, **5.9h**, and **5.9k**)

Table 0.5. Results of linear relationship between stomatal conductance and soil moisture. Shaprio-Wilks refers to the test for normality of residuals (i.e. if value is < 0.05 the data are not normal). Significant treatment effects are denoted with asterisks.

Drought Type	Species	Slope (g_s·SM)	Adjusted R^2	*P*-value	Shapiro-Wilks
All data	*Festuca*	22.31	0.238	< 0.001*	0.354
	Lolium	27.46	0.430	< 0.001*	0.079
	Phalaris	28.72	0.229	< 0.001*	0.119
Short	*Festuca*	17.34	0.328	< 0.001*	0.897
	Lolium	23.87	0.370	0.002*	0.452
	Phalaris	NS	0.010	0.264	0.262
Prolonged	*Festuca*	41.68	0.378	< 0.001*	0.961
	Lolium	38.81	0.580	< 0.001*	0.056
	Phalaris	52.17	0.483	< 0.001*	0.235
Repeated	*Festuca*	16.28	0.109	0.024*	0.362
	Lolium	23.59	0.251	< 0.001*	0.051
	Phalaris	26.88	0.210	0.002*	0.497

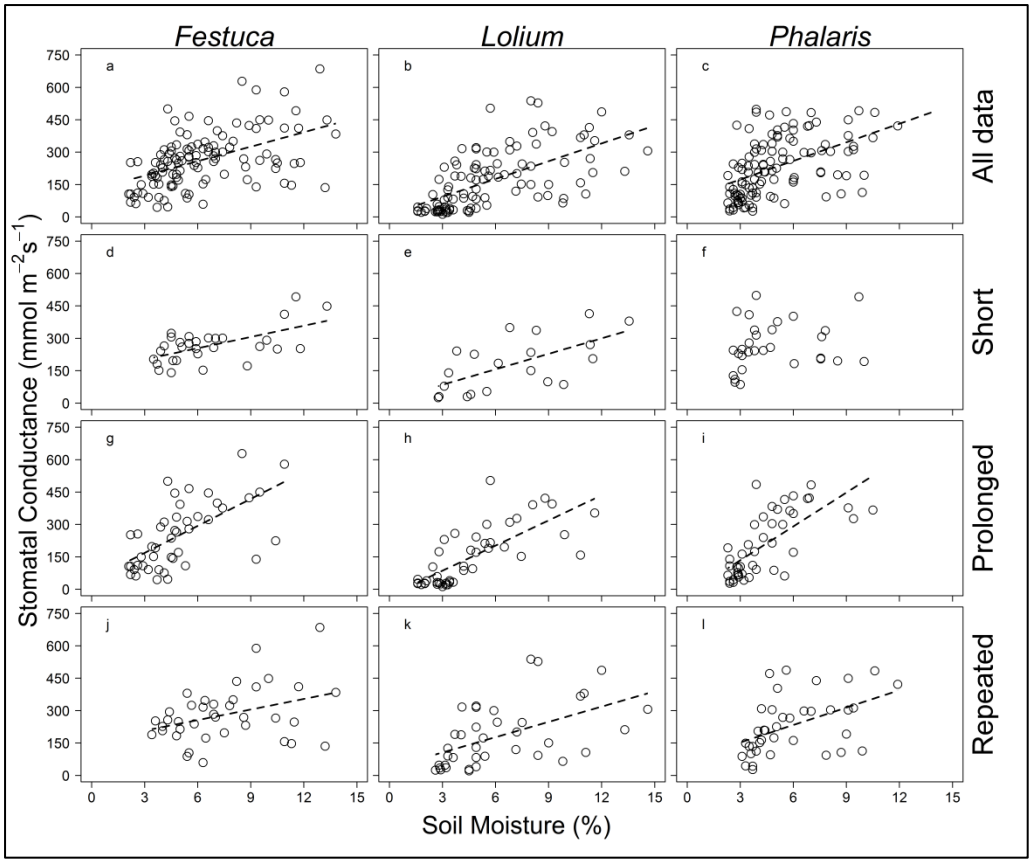

Figure 5.9. Linear relationships between soil moisture (x-axis) and stomatal conductance (y-axis) for each species (columns) and treatment (rows). The "All data" row is the combined data from the three drought treatments: short, prolonged, and repeated. Plots with dashed lines indicate a significant linear relationship of which the details are provided in Table 5.5.

5.4 DISCUSSION

In this study we subjected three common pasture grasses to three different types of drought, which varied in their intensity and timing, with species being grown in isolation and in mixtures to determine how competition may further affect productivity under future rainfall regimes. We found competition had negative effects on AGB production in *Phalaris* and *Lolium*, but that the extent of competitive suppression was unaffected by the severity and timing of drought. *Festuca*, however, showed a different pattern whereby competition was reduced during the short-term drought indicating periods under future rainfall scenarios where the utilisation of *Festuca* in pasture may be beneficial to promote AGB. We hypothesized that both the intensity and timing of drought would influence competition, but our results suggest that the length of drought had no significant effect on the outcomes of competition. However, the timing of drought was found to be important (i.e. greater competition in the repeated drought than the short drought). Our results indicate that the predicted shifts in rainfall regimes toward more variable rainfall (Knapp et al. 2008; Good et al. 2016) will not have consistent effects on interspecific interactions contributing to altered plant community dynamics. In the past, others have considered grouping plants by functional type in order to explain plant community responses to drought (Stampfli and Zeiter 2004; Fry et al. 2013a,b; Gherardi and Sala 2015); however, we observed unique responses across three mesic perennial grasses indicating that this may not be a robust approach.

We found that the stomatal conductance of *Festuca* was less responsive to declining soil moisture, suggesting this strategy may have reduced competition under short-term drought stress; however, the three species in this study have been observed to have comparable desiccation tolerances (White et al. 2001) and water-use efficiencies (Neal et al. 2011). Although water-use strategy could explain *Festuca*'s performance in this experiment, further data are required to adequately describe each species' explicit water-use strategy

(Sade et al. 2012). In addition, we were unable to separate BGB in mixtures, so *Festuca* may have had greater BGB in mixture than the remaining species (or a shift in root to shoot biomass), which is a common strategy to cope with water limitation (Karcher et al. 2008). These missing data represent some uncertainty in our study because belowground responses are expected to respond to altered soil water availability (Wang et al. 2018; Bristiel et al. 2018). It is also difficult to sort belowground material by species due to root entangling (e.g. Schwinning et al. 2017 using ~9 L pots and Montazeaud et al. 2018 using ~11 L pots) and we encountered the same issue using 45 L planter bags in our study.

We hypothesized that competition would be greatest during drought and would decrease following re-watering, but found this was true in only *Festuca*. The fact that *Festuca* experienced near zero competition during the short-term drought and upon re-watering (i.e. during recovery) could indicate it has a different strategy with regards the acquisition and use of water, compared to the remaining species. While the three species have comparable desiccation tolerances to drought (White et al. 2001) and water-use efficiencies (Neal et al. 2011), there could be other non-measured factors making *Festuca* superior. We did observe, however, that *Festuca* overall had minimal change in g_s given changes in soil moisture which could indicate a superior water-use strategy. Because of root entanglement in all treatments, we were unable to measure competitive effects on total and root biomass, which are likely to be affected by altered soil water availability (Wang et al. 2018; Bristiel et al. 2018). While the allocation of biomass is an important factor in drought response and tolerance, changes in morphology also occur (Zhou et al. 2018). If such changes occurred in *Festuca*, and not in the remaining species, this could also explain the observed patterns. Regardless of the mechanism, for pasture management aboveground, productivity for foraging opportunities was best in *Festuca* under specific scenarios and therefore this species is a good candidate to add in mixed-species pastures under future rainfall regimes.

While we could not test the direct effects of competition on belowground biomass, our data suggest that belowground competition occurred and is an important aspect of community response to future droughts. When looking at the total belowground biomass of mixtures compared to isolated individuals, in all drought types and harvesting periods, the amounts were comparable. This suggests that in a species mixture, one species, two species or all species underwent reduced belowground productivity as a result of having neighbours in most instances. In all scenarios we did observed root entanglement, indicating the belowground competition was occurring. Additionally, in all three drought treatments the amount of root biomass produced in mixture was comparable to the amount produced by individuals of each species. This was more pronounced upon re-watering; however, an exception may have occurred in the short drought during the resistance period. If competition was no occurring belowground during that period, it potentially revealed itself in the aboveground competitive effects data, where *Festuca* had near-zero effects of neighbouring species on growth. It is difficult to determine how our results may differ from those in the field, given rooting depth is typically restricted by nutrient/water limitation as soil depth increases; whereas in our study the Menangle soil was homogenized.

The duration of drought has been shown to affect the recovery rate of physiological activity, namely g_s (Miyashita et al. 2005). In our experiment, *Lolium* was the first species to have near-zero g_s and upon re-watering, had no biomass response under short-term drought. Under long-term drought, neither AGB nor BGB had a significant response to re-watering for *Lolium*, indicating lower recovery potential compared to *Festuca* and *Phalaris*. During the repeated drought, all species had significant increases in biomass following the second re-watering. This indicates, at least in *Lolium*, that the first drought may have generated a legacy effect and increased the capacity for plants to cope with the second drought. It should be noted in some cases the g_s in the plants assigned to the drought treatment were slightly higher

than those assigned to the control treatments, before the commencement of the soil water treatments. This could be due to differences in the microenvironment between rainfall exclusion shelters. The site typically receives wind moving from west to east, and the control plants were located in the southeast half of the shelters potentially having some shading effects through the day from the shelter's structural components. An additional limitation in this study is that we did not use an intraspecific competition control, which could change interpretation of the results (i.e. are competitive effects similar when the neighbour is the same species?).

5.5 CONCLUSIONS

In this study, we addressed two hypotheses regarding the impact of the intensity and timing of drought on competition between three common pasture species. We found that competition, manifest as reduced productivity due to the presence of neighbours, was ubiquitous across the experiment. *Festuca*, however, experienced minimal competition during mild drought stress and its recovery from short-term drought was both strong and unaffected by competition. Under future climate conditions, where larger and less frequent rainfall events are likely to occur, *Festuca* could maintain production and compensate for greater negative drought effects on co-existing species. The use of multispecies pastures may reduce the potential effects of changing rainfall patterns. This study highlights the importance of biotic interactions in potentially shaping pasture communities under future rainfall regimes.

Chapter 6: Synthesis and Conclusions

6.1 SYNTHESIS

Grassland ecosystems provide many ecosystem services, so developing a mechanistic framework to understand how they may respond to future climate change scenarios is crucial (Sala and Paruelo 1997; Duru et al. 2019). Since commencing this thesis in February 2016, record breaking heatwaves and prolonged, severe drought have affected a large portion of Australia, manifesting inas unprecedented impacts on the economy and ecology of the continent (Bureau of Meteorology). According to the Bureau of Meteorology, the 2018-2019 Australian summer was the hottest on record, with inland regions like Cobar and Broken Hill, NSW receiving 190 and 126 mm of rainfall in the last year, representing only 54.3 and 50.4% of their long-term annual totals, respectively (as of April 2nd 2019). Given the importance of precipitation for grassland productivity (Mowll et al. 2015; Griffin-Nolan et al. 2018), the unprecedented heat and very low rainfall have likely had dramatic consequences for Australian grasslands. In this thesis, I investigated the ecological consequences of altered rainfall regimes while developing a plant functional traits based framework to explore mechanisms and test hypotheses that allow me to apply new, mechanistic understanding to a wide range of grasslands world-wide. As observed in this thesis, traits and plant functional types provided tremendous power for understanding the responses of grassland communities to changes in rainfall. A brief summary of the overall objectives of the thesis is given below. In the thesis I aimed to address the current lack of knowledge regarding:

1) How do the abundance of traits, as surrogates for plant strategies, affect sensitivity of mesic grasslands to rainfall?
2) Which traits are important for physiological performance under drought and soil nutrient management (i.e. fertilisation)?

3) Can plant functional types be used to assess aboveground biomass in grassland and rangeland ecosystems?

6.1.1 Determine how the abundance of traits affects the sensitivity of productivity in a mesic grassland to rainfall variability (Chapter 2).

This thesis objective acknowledges that we have a comprehensive understanding of the precipitation-productivity relationship observed in grasslands worldwide (Sala 2001; Guo et al. 2012b; Wilcox et al. 2017; Wu et al. 2017), but a clear, mechanistic explanation for differing sensitivities to changes in the rainfall regimes is still lacking (Knapp et al. 2017). The use of traits was a constructive way to approach this issue, because functional traits represent universally-applicable surrogates for plant strategies that can be applied to ecosystems across the world (Reich 2014). In particular, given rainfall is a primary resource regulating grassland productivity, traits related to how this, and other key resources. are captured, stored and used was key to address this topic (Grime et al. 1997). Specifically, I hypothesized that a greater abundance of plant species with a more *acquisitive* strategy within a community (i.e. biomass-ratio hypothesis) - related to rapid growth and quick tissue-turnover - would be associated with a relatively high level of sensitivity of productivity to above- or below average rainfall amounts. Conversely, an abundance of *conservative* plant strategies in a community would confer stability to the community, as these species use resources more slowly and independent of daily/seasonal fluctuations in the environment.

In our mesic grassland, we found that community-weighted abundances of specific leaf area, specific root length, foliar C:N and stem density predicted rainfall-driven variation in productivity. While this was only a single experiment, we could consider applying this finding to other grassland ecosystems worldwide. For example, we could compare these results to two theoretical plant communities: an annual-dominated community and a

perennial tussock grassland in semi-arid environments. An annual-dominated community, comprised primarily of species with *acquisitive* strategies would undergo rapid increases in productivity under above-average rainfall conditions. However, the same community would experience minimal productivity during periods with below-average rainfall. In total, the change in productivity would be high for a given change in rainfall. If the same rainfall patterns were to occur in a perennial tussock grassland, there would be foliar die-back of the grasses in the community, although a larger proportion of the standing biomass would remain. This would result in more stable productivity over time because of the abundance of conservative traits in the community.

The idea of species' functional attributes contributing to productivity and sensitivity to environmental change is not unique (Cadotte et al. 2009; Roscher et al. 2012); however, few studies have applied this knowledge in the context of global change (Griffin-Nolan et al. 2018). In addition, few studies have considered the role that PFT variability (intraspecific variation) has in mediating the responses of plant communities to environmental change (Kichenin et al. 2013). However, some studies found trait variability to be an important explanatory factor in plant community responses (Jung et al. 2014). In our study, we found that variation in traits was also important in understanding grassland sensitivity to rainfall, although variation in trait values weren't necessarily correlated to the *acquisitive*/*conservative* values of the trait itself.

In conclusion, in this chapter I investigated the role of traits in understanding the sensitivity of productivity to inter-annual rainfall amounts in a mesic grassland. The relative dominance of *acquisitive* or *conservative* strategies was found to be a useful predictor of sensitivity in productivity. In addition, I found that intraspecific variation in traits may be related to plant resource-use strategy, and therefore predictive of ecosystem response to environmental variation in the future.

6.1.2 Determine the utility of traits as indicators of plant physiological performance to extreme drought and soil nutrient management (i.e. fertilisation) in a mesic grassland (Chapter 3).

In this chapter, we investigated the physiological and productivity responses of dominant grasses in a mesic grassland to drought and soil P fertilisation, and linked the observations to underlying traits. The goal was to better understand the usefulness of traits as predictors of species' performance under future rainfall regimes and management. We found reduced rainfall decreased aboveground productivity by 29 % and P addition increased productivity by 62 %. However, the benefit of P addition did not offset the negative impact of drought. Others have used plant functional types (e.g. grass *vs*. forb *vs*. legume) to explain changes in community productivity and diversity under experimental rainfall manipulation (Stampfli and Zeiter 2004; Fry et al. 2013; Gherardi and Sala 2015); we, however, observed unique responses across mesic perennial grasses with respect to performance and traits. This result highlights the complexity of predicting vegetation responses to climate change and management based on coarse groupings. In fact, we found the organ-level trait, root tissue density, to be a key driver of species' responses within the experiment. More specifically, root tissue density was the trait most closely associated with productivity and recovery of stomatal conductance following late summer rainfall events. While the link between root morphology and stress/drought tolerance has been observed previously (Huang 2000; Wahl and Ryser 2000; Louise Comas et al. 2013), few studies have measured interspecific responses of such traits across perennial grasses.

Overall I found productivity of the system to be sensitive to both rainfall and phosphorus treatments, indicating a potentially negative outlook given future climate change scenarios. A particularly interesting result was that the species most sensitive to reduced rainfall, *Setaria parviflora*, was also the only species to have its traits unaffected by rainfall

2232 or P addition. This could indicate that a lack of plasticity of traits could be a useful indicator
2233 of how environmental change will affect species' performance (Couso and Fernández 2012;
2234 Kramer et al. 2018). Following the conclusion of *Chapter 2*, I hypothesized that intraspecific
2235 variability of traits could be a useful indictor of species-level responses to abiotic stress (e.g.
2236 Jung et al. 2014), and our results in this experiment corroborate that assumption. In addition,
2237 root tissue density in *Cynodon dactylon* became increasingly *conservative* with P addition,
2238 which was correlated with greater productivity under drought. This provides further evidence
2239 that shifts in trait values are inherently linked to stress tolerance (Romero-Munar et al. 2018).
2240 However, further work is required as shifts in trait values were not uniform across all species
2241 (i.e. not all positive or negative across species and treatments).

2242 Based on the physiological data measured in this study (stomatal conductance (g_s)),
2243 we found positive effects of P addition under drought, indicating the potential for P addition
2244 to mitigate negative impacts of future rainfall patterns. However, this enhanced recovery of g_s
2245 did not translate to greater productivity during the timeframe over which species were
2246 assessed. Others have observed the physiological benefits of P addition in plants under stress
2247 (dos Santos et al. 2006), but our study was relatively short in duration and occurred in a
2248 community that showed minimal indication of P limitation (i.e. no effect on community
2249 productivity over several years of fertilisation) (Bennett and Adams 2001).

2250 While P addition had minimal direct effect on productivity, with the exception of
2251 *Cynodon dactylon*, P fertilisation did result in changes in root traits (tissue density and
2252 specific root length), and these were positively associated with productivity. This result, in
2253 tandem with observations of faster recovery of g_s, suggests that P addition may alleviate the
2254 negative impacts of drought under some scenarios, specifically where co-limitation occurs or
2255 where P addition causes shifts in traits conducive to performance under drought (Ho et al.
2256 2004; Comas et al. 2013; Fort et al. 2015).

6.1.3 Assess the ability to utilise plant functional types to assess aboveground biomass in grassland and rangeland ecosystems in remote locations (Chapter 4).

Plant productivity is a primary function of plant communities, associated with carbon sequestration and nutrient cycling (Huxman et al. 2004). In this chapter, we addressed whether grouping plants based on their broad morphological characteristics is beneficial to quantifying plant biomass across the remote rangelands of eastern Australia (Nafus et al. 2009; Paul et al. 2016). As observed by others, the logistics of obtaining accurate plant biomass estimates in remote settings is difficult (Flombaum and Sala 2007). The development of allometric equations, using non-destructive proxies for biomass, is common practice; however, issues around the development of these remain. Specifically, questions exist around what attributes should be measured as correlates with biomass (Ishihara et al. 2015). In addition, are species-specific equations necessary to accurately estimate biomass or can species can be grouped together (Nafus et al. 2009; Paul et al. 2016)? In this study, we addressed these questions using 15 plant species from eastern Australia's rangelands.

We found using plant height, in addition to cover, was marginally superior to cover alone in predicting plant biomass of the 15 species surveyed. This trend has been observed by others (Huenneke, Clason, and Muldavin 2001) and may be beneficial when applying allometric equations across years (Ónodi et al. 2017). Others have found the addition of height to add minimal benefit to cover-based biomass estimates (Andariese 1986; Assaeed 1997; Guevara, Gonnet, and Estevez 2002; Nafus et al. 2009); Our results contradict this, but only marginally. Height is relatively easy to measure, so it should be considered as a candidate metric, along with plant cover, for allometric assessments of rangeland species in the future.

With respect to the use of species-specific and multispecies biomass estimates, others have found multispecies estimates to be more robust than species-specific estimates (Roxburgh et al. 2015; Chojnacky, Heath, and Jenkins 2013; Paul et al. 2016). Changes in rainfall patterns are likely to have strong effects on plant productivity in more arid environments, so the need to address these issues will become crucial in the future to estimate global carbon dynamics (Mowll et al. 2015; Ahlström et al. 2015; Haverd, Smith, and Trudinger 2016; Nolan et al. 2018). We conclude that adding a greater number of species, using additional non-destructive proxy measures (e.g. leaf and stem density) for prediction, and adding data across multiple years, are necessary to accurately estimate carbon stocks and ecosystem sensitivity to external drivers of change in the shrub- and herbaceous-dominated plant communities that characterize the eastern Australian rangelands.

6.1.4 Determine how reduced soil water availability, associated with drought, affect interspecific interactions (competition/facilitation) among three important pasture grasses (Chapter 5).

Resource limitation is a primary cause for competition amongst plant species (Harpole et al. 2016; Nathan et al. 2016). Projected changes in rainfall regimes predict greater soil water limitation in the future with likely impacts on plant community dynamics (Alan K. Knapp et al. 2008). Therefore, understanding how species interact under these novel conditions is crucial to managing grasslands and pastures worldwide (Bolger, Rivelli, and Garden 2005; Cullen et al. 2009). *Phalaris aquatica*, *Festuca arundinacea* and *Lolium perenne* are three of the primary

pasture species around the world and are very important in Australia (Reed 1996; Ayres et al. 2000; Clark et al. 2016). In this chapter, we addressed how the intensity and timing of drought affected competition amongst three economically important grass species.

We found competition to be ubiquitous across drought types (short, prolonged, repeated) both in terms of its effect on plant resistance to (and recovery from) drought (i.e. before or after re-watering). However, there were instances when competition had minimal negative impacts on productivity in *Festuca*. Particularly, during mild drought stress and associated drought recovery, competition had negligible effects on *Festuca*'s productivity, which suggests that it will maintain productivity in mixed species grasslands, especially if rainfall becomes more unpredictable. The three species used in this study have previously been observed to have similar drought tolerances and water-use strategies (White, Campbell, and Kemp 2001; Neal, Fulkerson, and Sutton 2011). Our studies indicate, however, that in mixture *Festuca* was superior under certain conditions. While minimal experimental evidence exists regarding plant-plant interactions under future climate change scenarios (Grant et al. 2014; Ploughe et al. 2018), our study suggests that the increasing limitation of soil moisture availability will not affect plant-plant interactions uniformly, thus justifying further experiments in this area.

6.2 CONCLUSIONS

6.2.1 General Conclusions

Changes in rainfall are likely to affect grassland ecosystems. The use of plant functional traits will increase mechanistic understanding of plant response to changes in the environment. Firstly, we observed in Chapters 2 and 3 the importance of *acquisitive* and *conservative* strategies on the performance, sensitivity, and physiological response of grassland species to

varying rainfall amounts and soil nutrient management. In the future, such data should be used for multi-site analyses across longer timescales to determine the validity of these findings. Already, the importance of using traits for global change ecology has been identified (Griffin-Nolan, et al. 2018); however, much work is needed to adequately address trait use in grasslands across biomes. Second, in order to better understand how traits affect key ecosystem processes, the development of allometric assessments may be necessary in order to rapidly quantify standing biomass and changes thereafter. Finally, understanding the consequences of water-limitation associated with droughts will affect multispecies communities in unpredictable ways. Considerable work is needed to adequately predict such changes, although recent frameworks may provide guidance on how to address such topics (Grant et al. 2014; Ploughe et al. 2018).

6.2.2 Grasslands in Australia

Given the observed trends in rainfall and temperature over the last few years (BOM; www.bom.gov.au) and predictions for the future (Easterling et al. 2000; Evans et al. 2017), in addition to an increased demand for livestock production (Kemp 2017) and rapid land-use change (Williams et al. 2015), grassland ecosystems will be substantially impacted. Grasslands play a critical role in providing ecosystem services, and adequately managing grassland biodiversity and resilience to climatic events will be central to preserving their function. Based on the results of this thesis, key insights into the future of grasslands under climate change and management approaches can be developed to mitigate these impacts.

Firstly, managing community-level trait composition can be used to moderate the resistance or resilience to changes in rainfall. A greater abundance of conservative species will mitigate the impacts of drought; however, once a stress-tolerance threshold is exceeded, the loss of species with these strategies could inhibit the rate of subsequent ecosystem

recovery. Conversely, a high abundance of *acquisitive* species will result in high sensitivity to changes in rainfall amounts, but also a greater ability to recover once conditions are favourable again. In addition, soil nutrient management should focus on creating shifts in traits conducive to drought tolerance, rather than focusing on maximizing productivity. While the latter is typically the goal of fertilisation, and contributes to carbon sequestration, maintaining productivity in the face of increasingly variable future rainfall regimes will not be possible if traits shift towards less tolerant strategies.

Management of PFTs and diversity in grasslands and pastures can be achieved in two ways. During formation of pastures or rehabilitation/restoration of grasslands, appropriate species compositions can be potentially sown to maximize functional diversity to mitigate negative effects of future climatic events. The second way is through targeted grazing (Álvarez-Martínez, Gómez-Villar, and Lasanta 2016). Palatability of different plants species is unique between type of domestic/native grazers, and therefore research should focus on interim measures to promote functional diversity. Furthermore, seasonality and sequences of rainfall events can promote periods of high- or low growth for individual species in mixed communities. Thus, grazing can be targeted during these periods to promote some species over others.

6.3 RECOMMENDATIONS

Based on the results and observations, here are a few recommendations of topics which should be investigated and how they may fit into the scope of research explored in this thesis. In order to accurately understand how grasslands communities will respond to climatic extremes, fewer studies conducting in-field manipulations of rainfall should occur. In fact, the costs should be invested in long-term monitoring efforts in natural systems. Since the

colonization of Australia, the continent has not encountered the full severity of drought conditions that have been observed in the last 1000 years (Vance et al. 2015). Thus, the current definition of extreme (i.e. 1-in-100 years, 99th percentile of events) implemented in the grassland-drought literature (M. D. Smith 2011) is inadequate given the occurrence of megadroughts which can occur in Australia. Furthermore, monitoring efforts would enhance forecasting abilities in Australia generally. For example, the nearest weather station to my field site in Quilpie, QLD was ~150 km away. Enhanced monitoring efforts would close these gaps to better understand climatic drivers of plant productivity at the local and continental scale.

An additional research need is to investigate how grazing, in tandem with interannual patterns in rainfall interact to shape grassland ecosystems. Mowing or clipping biomass for quantification purposes to not adequately reflect the effects of grazing. Grazing is selective and thus has unique effects on species composition from site-wide mowing (Moog et al. 2002; Tälle et al. 2016). Such selective processes will likely have effects on competition as well, representing unique filters on species composition which are not typically quantified in the applied pastures research.

Appendix and Supplementary Data

Supplementary Table S2.1. List of species found at the field site from 2014 to 2018, their contribution to total aboveground net primary productivity (ANPP), and the cumulative ANPP contribution. Asterisks denote species used in the functional trait analysis. Data include life cycle, family, photosynthetic pathway, and their origin.

Species	Total ANPP Contribution 2014-2018	Cumulative ANPP Contribution	Life Cycle	Family	Photo	Origin
Cynodon dactylon	61.4%	61.4%*	Perennial	Poaceae	C4	Non-native
Setaria parviflora	17.3%	78.7%*	Perennial	Poaceae	C4	Non-native

Microlaena stipoides	11.8%	90.54*	Perennial	Poaceae	C3	Native
Eragrostis curvula	2.8%	93.33*	Perennial	Poaceae	C4	Invasive
Commelina cyanea	1.7%	95.0%*	Perennial	Commelinaceae	C3	Native
Hypochaeris radicata	1.1%	96.1%	Annual	Asteraceae	C3	Non-native
Axonopus fissifolius	1.0%	97.2%	Perennial	Poaceae	C4	Non-native
Juncus species	0.9%	98.1%	Perennial	Juncaceae	C3	Unknown
Sonchus oleraceus	0.5%	98.6%	Annual	Asteraceae	C3	Non-native
Cyperus sesquiflorus	0.4%	99.0%	Perennial	Cyperaceae	C4	Non-native
Wahlenbergia species	0.3%	99.3%	Annual	Campanulaceae	C3	Native
Lotus corniculatus	0.3%	99.6%	Perennial	Fabaceae	C3	Native
Dichelachne crinita	0.2%	99.8%	Perennial	Poaceae	C3	Native
Bidens species	<.1%	99.9%	Annual	Asteraceae	C3	Non-native
Moss (unknown)	<.1%	99.9%	Perennial	Unknown	C3	Unknown
Sporobulus species	<.1%	99.9%	Perennial	Poaceae	C4	Unknown
Plantago lanceolata	<.1%	99.9%	Perennial	Plantaginaceae	C3	Non-native
Paspalum species	<.1%	99.9%	Perennial	Poaceae	C4	Non-native
Vicia sativa	<.1%	99.9%	Annual	Fabaceae	C3	Non-native
Lobelia purpurascens	<.1%	99.9%	Perennial	Campanulaceae	C3	Non-native
Oxalis corniculata	<.1%	99.9%	Perennial	Oxalidaceae	C3	Non-native
Opercularia species	<.1%	100.0%	Perennial	Rubiaceae	C3	Unknown

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Supplementary Table S2.2. Soil total carbon, nitrogen and phosphorus concentrations in March 2016 and 2017 in the four rainfall and phosphorus treatments.

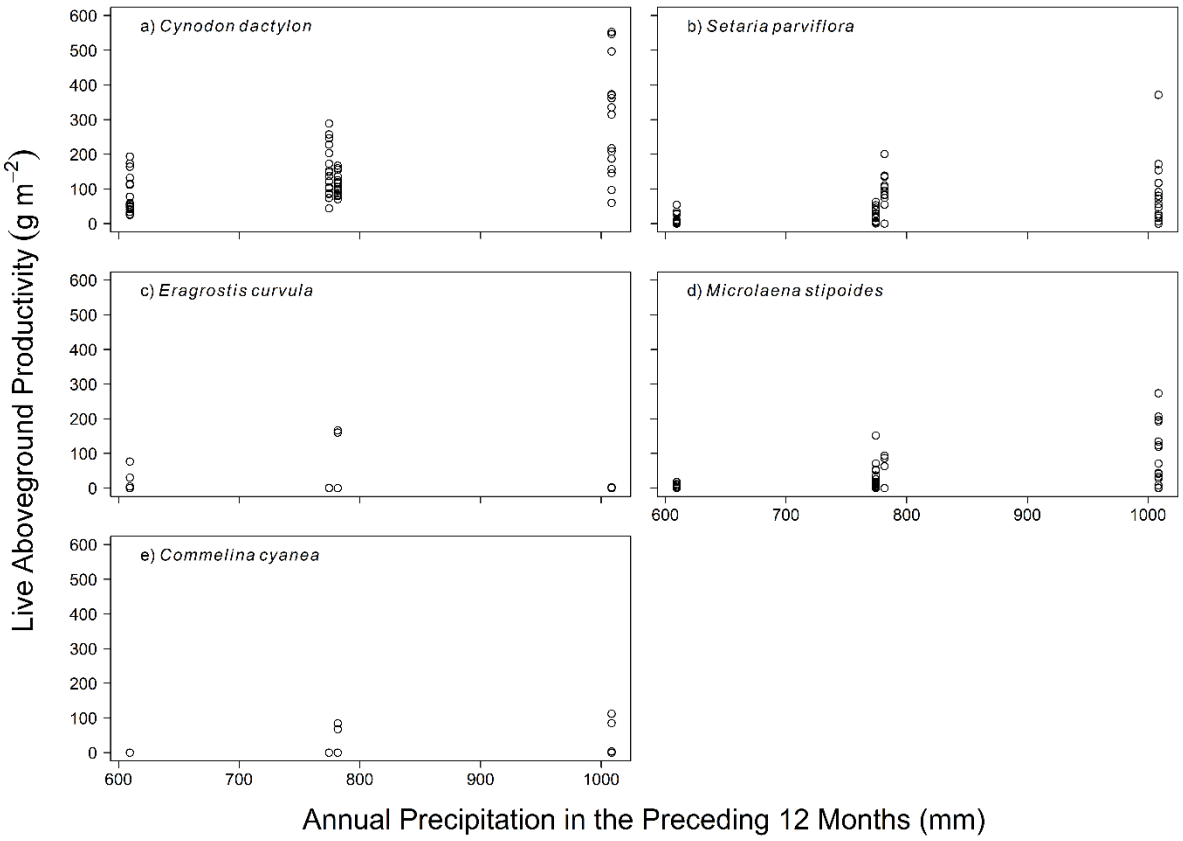
Treatment	March 2016			March 2017		
	C (µg g^{-1})	N (µg g^{-1})	P (µg g^{-1})	C (µg g^{-1})	N (µg g^{-1})	P (µg g^{-1})
Ambient	6750 ± 380	610 ± 30	180 ± 10	8980 ± 410	700 ± 50	300 ± 30
P added	7370 ± 85	660 ± 70	250 ± 20	9650 ± 760	730 ± 30	520 ± 120
Shelter	8720 ± 63	800 ± 90	210 ± 30	9880 ± 770	820 ± 80	230 ± 30
Shelter + P	8460 ± 66	710 ± 60	300 ± 50	10600 ± 1630	860 ± 170	370 ± 90

Supplementary Table S3.1. Summary statistics for the effects of rainfall and phosphorus treatments, and their interaction, from linear mixed models using Kenward-Roger Degrees of Freedom Approximation. Highlighted columns are those with significant effects for ease of reading. Abbreviations: ANPP = aboveground net primary productivity (g m^{-2} y^{-1}), SLA = specific leaf area (mm^2 mg^{-1}), FolCN = foliar C to N ratio, LDMC = leaf dry matter content (mg g^{-1}), HT = height (mm), RTD = root tissue density (mg mm^{-3}), DIA = root diameter (mm), SRL = specific root length (cm g^{-1}), RBI = root branching intensity (branch #·cm).

Response Variable	**Group/Species**	**Rainfall**		**Phosphorus**		**Rain*Phosphorus**	
		F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
ANPP	Total Live	8.42	0.018*	16.16	0.003*	3.30	0.103
	C. dactylon	1.63	0.234	14.85	0.004*	1.64	0.232
	E. curvula	1.41	0.266	0.43	0.529	0.55	0.479
	M. stipoides	0.59	0.461	0.90	0.367	6.10	0.036*
	S. parviflora	15.80	0.003*	0.14	0.719	0.12	0.733
	Non-dominant	0.00	0.972	0.68	0.431	1.44	0.260
SLA	*C. dactylon*	0.02	0.883	1.19	0.279	0.00	0.987
	E. curvula	0.788	0.377	7.04	0.009*	0.30	0.583
	M. stipoides	10.37	0.002*	0.07	0.794	0.47	0.497
	S. parviflora	0.03	0.855	3.59	0.062	2.78	0.099
FolCN	*C. dactylon*	1.48	0.235	3.36	0.079	2.89	0.101
	E. curvula	0.27	0.606	0.04	0.846	0.16	0.696
	M. stipoides	0.02	0.888	0.10	0.752	0.10	0.756
	S. parviflora	0.00	0.955	0.48	0.494	0.16	0.694
LDMC	*C. dactylon*	0.09	0.769	1.97	0.168	0.41	0.525
	E. curvula	1.68	0.202	0.74	0.395	0.502	0.483
	M. stipoides	0.02	0.887	1.26	0.271	0.00	0.942
	S. parviflora	1.57	0.217	0.382	0.540	1.13	0.293
HT	*C. dactylon*	0.03	0.869	42.55	< 0.001*	1.43	0.237
	E. curvula	0.08	0.782	0.00	0.946	2.27	0.138
	M. stipoides	10.38	0.003*	0.02	0.890	0.687	0.413
	S. parviflora	2.62	0.111	0.00	0.977	1.01	0.318
RTD	*C. dactylon*	1.91	0.207	38.74	< 0.001*	0.616	0.456
	E. curvula	1.34	0.277	14.03	0.005*	0.00	0.973
	M. stipoides	0.977	0.356	5.95	0.045*	0.05	0.823
	S. parviflora	0.00	0.979	0.86	0.377	0.07	0.803
DIA	*C. dactylon*	1.83	0.214	1.14	0.319	0.14	0.717
	E. curvula	2.38	0.157	2.82	0.127	0.70	0.425
	M. stipoides	0.76	0.411	0.22	0.650	2.83	0.129
	S. parviflora	0.03	0.860	1.23	0.297	0.02	0.901
SRL	*C. dactylon*	1.05	0.338	59.84	< 0.001*	0.32	0.590
	E. curvula	8.75	0.016*	2.99	0.118	0.08	0.784
	M. stipoides	0.30	0.599	13.17	0.008*	0.11	0.753
	S. parviflora	0.02	0.902	1.26	0.292	0.13	0.727
RBI	*C. dactylon*	0.71	0.428	161.04	< 0.001*	0.07	0.802
	E. curvula	3.73	0.086	0.14	0.718	1.17	0.308
	M. stipoides	4.54	0.069	15.35	0.005*	0.63	0.448
	S. parviflora	4.24	0.070	0.11	0.748	2.67	0.119

Supplementary Table S3.2. ANOVA results for stomatal conductance by species and treatment. *P*-values in bold indicate significant treatment effects.

Species	Trt	Day 1		Day 3		Day 6		Day 14		Day 23		Day 31		Day 38	
		F-stat	*P*-val	F-stat	*P*-val	F-stat	*P*-val	F-stat	*P*-val	F-stat	*P*-val	F-stat	*P*-val	F-stat	*P*-val
C. dactylon	Rain	19.98	**0.002**	122.20	**< 0.001**	24.51	**0.001**	20.23	**0.002**	137.26	**< 0.001**	174.47	**< 0.001**	3.49	0.099
	Phos	13.18	**0.007**	49.77	**< 0.001**	14.68	**0.005**	5.74	**0.044**	55.81	**< 0.001**	48.27	**< 0.001**	8.03	0.022
	R*P	23.04	**0.001**	29.36	**< 0.001**	9.42	**0.015**	2.06	0.189	28.18	**< 0.001**	20.18	**0.002**	0.31	0.596
E. curvula	Rain	1.99	0.210	28.09	**< 0.001**	15.18	**0.005**	157.06	**< 0.001**	61.00	**< 0.001**	12.11	**0.008**	19.39	0.061
	Phos	1.86	0.196	0.25	0.634	6.78	**0.031**	130.02	**< 0.001**	37.80	**< 0.001**	6.45	0.035	4.73	0.002
	R*P	0.02	0.886	1.71	0.228	0.28	0.614	0.008	0.008	2.10	0.186	0.54	0.485	0.24	0.635
S. parviflora	Rain	19.50	**0.004**	97.92	**< 0.001**	11.03[1]	**0.016**	40.43	**< 0.001**	771.50	**> 0.001**	169.82	**< 0.001**	51.56	< 0.001
	Phos	16.37	**0.002**	2.70	0.139	9.31[1]	**0.011**	13.50	**0.006**	16.02	**0.004**	23.70	**0.001**	2.23	0.174
	R*P	13.14	**0.008**	3.33	0.106	0.45[1]	0.520	2.05	0.190	0.739	0.415	0.00	0.981	3.68	0.091
M. stipoides	Rain	NA	NA	NA	NA	63.21	**< 0.001**	28.50	**< 0.001**	67.28	**< 0.001**	411.49	**< 0.001**	9.86	0.014
	Phos	4.71	0.096	29.38	**0.006**	51.84	**< 0.001**	20.67	**0.002**	30.12	**< 0.001**	74.64	**< 0.001**	14.95	0.005
	R*P	NA	NA	NA	NA	1.70	0.229	0.76	0.409	2.65	0.142	20.22	0.002	0.78	0.404



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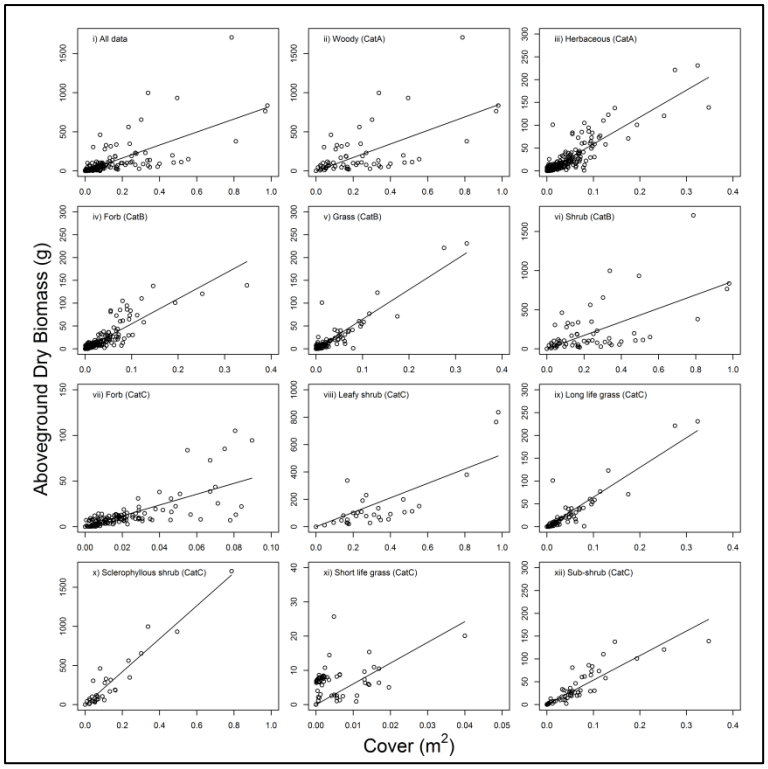
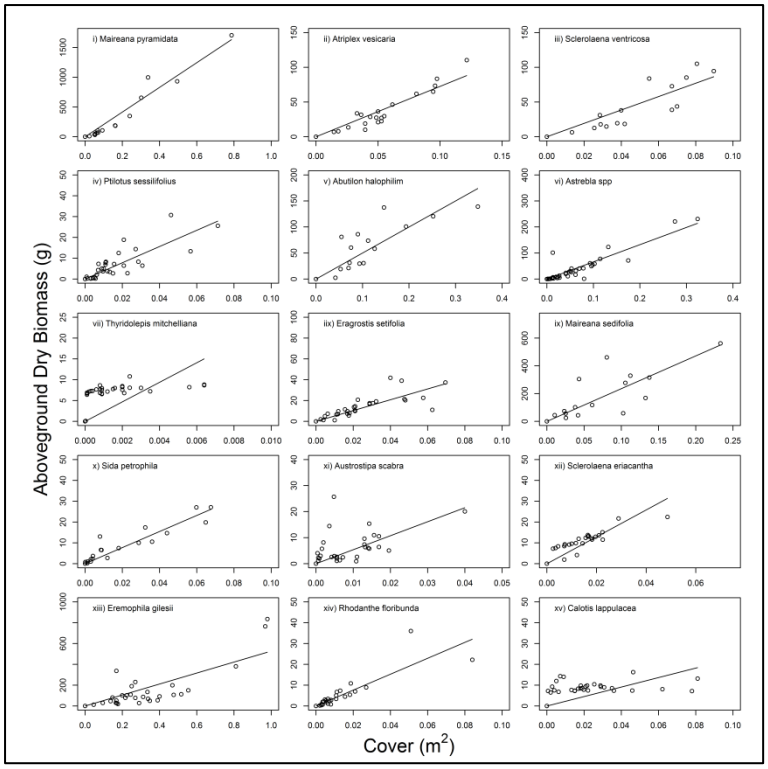
2421 **Supplementary Figure S2.1**. Plot specific productivity by species from 2014-2017.



Supplementary Figure S4.1. Scatter plot and regression line of the aboveground dry biomass and *cover* relationship for each of the groups sampled.



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2427 **Supplementary Figure S4.2**. Scatter plot and regression line of the aboveground dry

2428 biomass and *cover* relationship for each of the groups sampled.

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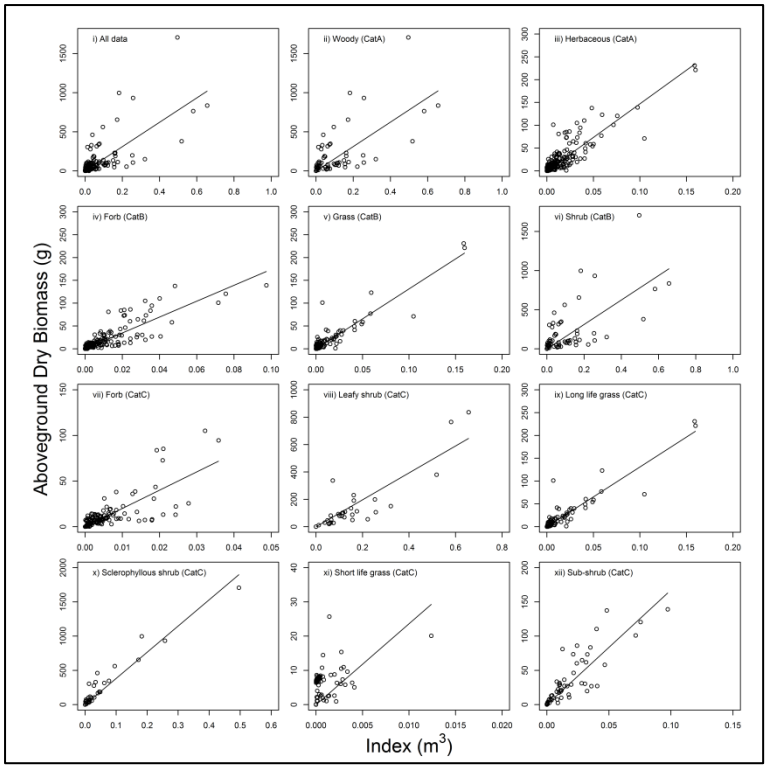

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2431 **Supplementary Figure S4.3**. Scatter plot and regression line of the aboveground dry

2432 biomass and *cover·height index* relationship for each of the groups sampled.

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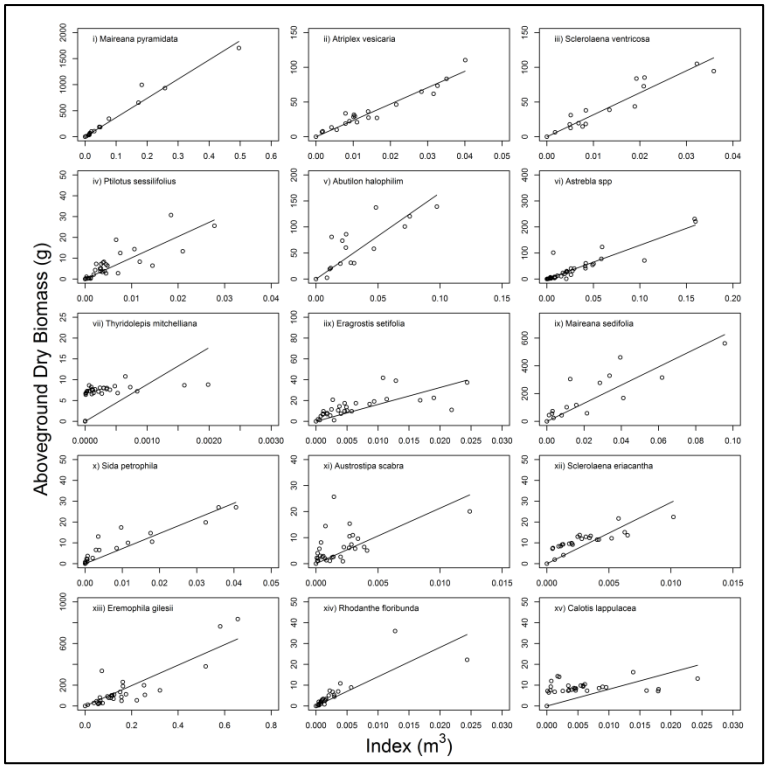

2434

2435 **Supplementary Figure S4.4**. Scatter plot and regression line of the aboveground dry

2436 biomass and *cover·height index* relationship for each of the groups sampled**.**

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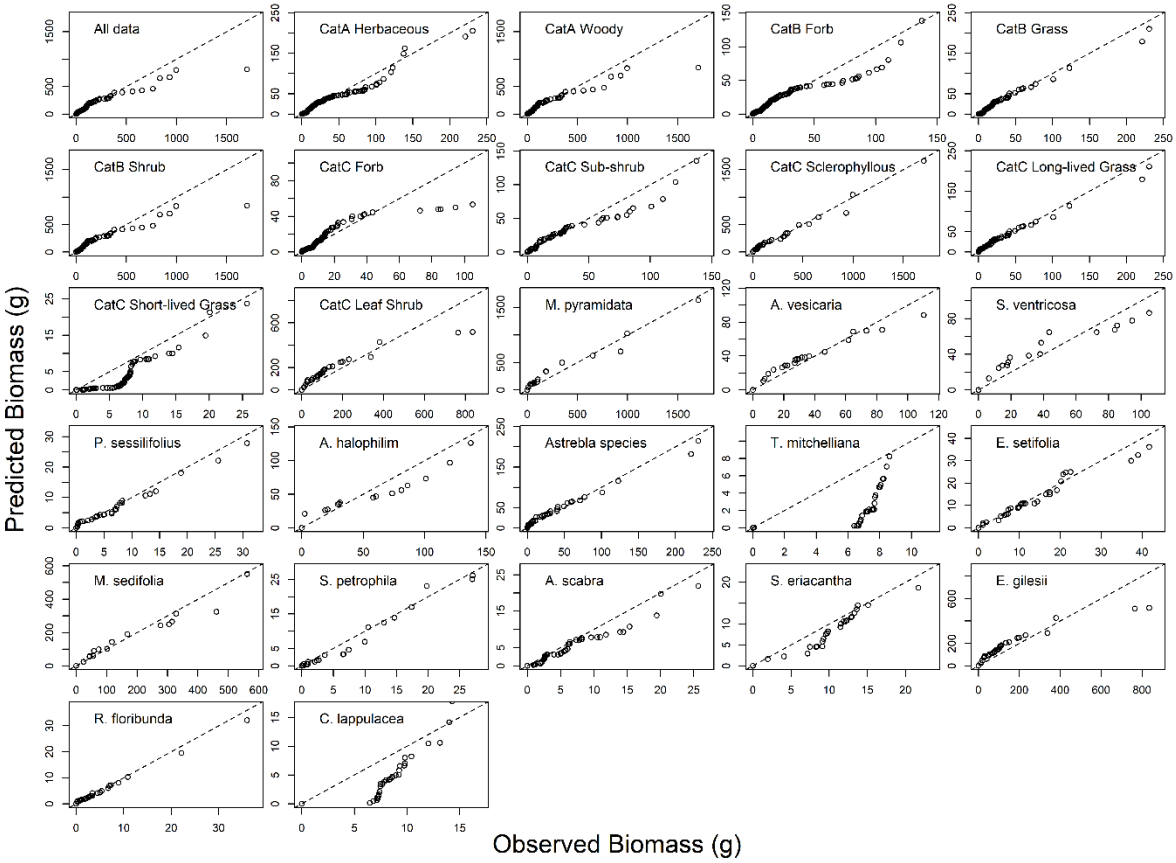

Table S4.5. Observed and predicted values of aboveground biomass using *cover* for allometric regressions.

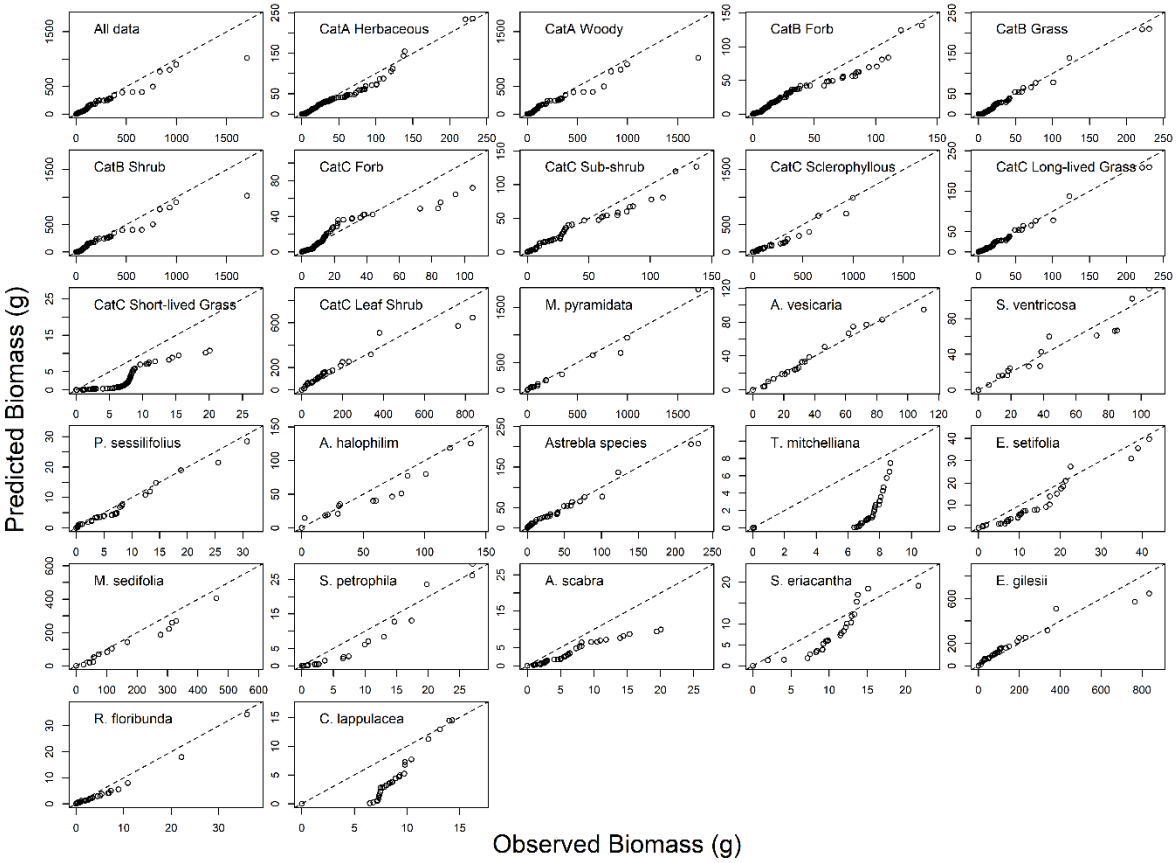

Figure S4.6. Observed and predicted values of aboveground biomass using *cover·height index* for allometric regressions.

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